UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Note to Reader

Background: As part of its effort to involve the public in the implementation of the Food Quality Protection Act of 1996 (FQPA), which is designed to ensure that the United States continues to have the safest and most abundant food supply. EPA is undertaking an effort to open public dockets on the organophosphate pesticides. These dockets will make available to all interested parties documents that were developed as part of the U.S. Environmental Protection Agency's process for making reregistration eligibility decisions and tolerance reassessments consistent with FQPA. The dockets include preliminary health assessments and, where available, ecological risk assessments conducted by EPA, rebuttals or corrections to the risk assessments submitted by chemical registrants, and the Agency's response to the registrants' submissions.

The analyses contained in this docket are preliminary in nature and represent the information available to EPA at the time they were prepared. Additional information may have been submitted to EPA which has not yet been incorporated into these analyses, and registrants or others may be developing relevant information. It's common and appropriate that new information and analyses will be used to revise and refine the evaluations contained in these dockets to make them more comprehensive and realistic. The Agency cautions against premature conclusions based on these preliminary assessments and against any use of information contained in these documents out of their full context. Throughout this process, If unacceptable risks are identified, EPA will act to reduce or eliminate the risks.

There is a 60 day comment period in which the public and all interested parties are invited to submit comments on the information in this docket. Comments should directly relate to this organophosphate and to the information and issues available in the information docket. Once the comment period closes, EPA will review all comments and revise the risk assessments, as necessary.

These preliminary risk assessments represent an early stage in the process by which EPA is evaluating the regulatory requirements applicable to existing pesticides. Through this opportunity for notice and comment, the Agency hopes to advance the openness and scientific soundness underpinning its decisions. This process is designed to assure that America continues to enjoy the safest and most abundant food supply. Through implementation of EPA's tolerance reassessment program under the Food Quality Protection Act, the food supply will become even safer. Leading health experts recommend that all people eat a wide variety of foods, including at least five servings of fruits and vegetables a day.

Note: This sheet is provided to help the reader understand how refined and developed the pesticide file is as of the date prepared, what if any changes have occurred recently, and what new information, if any, is expected to be included in the analysis before decisions are made. **It is not meant to be a summary of all current information regarding the chemical.** Rather, the sheet provides some context to better understand the substantive material in the docket (RED chapters, registrant rebuttals, Agency responses to rebuttals, etc.) for this pesticide.

Further, in some cases, differences may be noted between the RED chapters and the Agency's comprehensive reports on the hazard identification information and safety factors for all organophosphates. In these cases, information in the comprehensive reports is the most current and will, barring the submission of more data that the Agency finds useful, be used in the risk assessments.

Jack E. Housenger, Acting Director
Special Review and Reregistration Division

CANCER ASSESSMENT DOCUMENT # 2

REPORT of the 12-APRIL-2000 MEETING

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

MALATHION

28-APRIL-2000

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: Brian Dementi, Toxicologist

Marion Copley, Toxicologist

DOCUMENT PREPARATION: Marion Copley, Toxicologist

COMMITTEE MEMBERS IN ATTENDANCE: AT THE 12-APRIL-2000 MEETING (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam, Chairman

Karl Baetcke

Marion Copley

Vicki Dellarco

Sanju Diwan

Virginia Dobozy

Richard Hill

Mike Ioannou

Nancy McCarroll

Tim McMahon

Esther Rinde

Jess Rowland

Joycelyn Stewart

Clark Swentzel

Linda Taylor

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John Pletcher, Pathology Consultant

OTHER NON-COMMITTEE MEMBERS IN ATTENDANCE J. Carley, P. Deschamp, P. Fenner-Crisp, W. Hersey, P. Moe, B. Shackleford, M. Stasikowski, B. Tarplee and P. Wagner.

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CONTENTS

EXECUTIVE SUMMARY

On 24-September, 8-October, 15-October-1997, 10-June-1998, 24-February-1999 and 23-June-1999, the Health Effects Division's Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of malathion. The Committee reviewed the following studies: 1) Carcinogenicity study in B6C3F1 mice; 2) Combined chronic toxicity/carcinogenicity study in Fischer 344 rats with malathion; and 3) Combined chronic toxicity/carcinogenicity study with malaoxon, the active cholinesterase inhibiting metabolite of malathion in F344 rats. Relevant subchronic, chronic and mutagenicity studies were also reviewed at these meetings, as well as the results of the studies conducted with malathion and/or malaoxon (during 1978-80) by the National Cancer Institute/National Toxicology Program (NCI/NTP). On 12-April-2000, the CARC met to evaluate: 1) a new Pathology Working Group (PWG) report submitted by Cheminova on the female Fischer 344 rat liver tumors; 2) two issues raised by Dr. Dementi regarding the evaluation of malathion (items #4–mononuclear cell leukemia in Fischer 344 male rats and #7–oral tumors in Fischer 344 female rats from Attachment 25; 3) the 29-March-2000 letter from Jellinek, Schwartz & Connally, Inc. to Patricia Moe, "Re: Comments on EPA's Risk Assessments for Malathion;" and 4) discuss the weight of evidence and cancer classification for malathion based on the previously listed information.

The CARC #2 report supercedes the 2-February-2000 CARC (CARC #1) report. It contains a combined summary of the CARC #1 report and the CARC meeting of 12-April-2000. CARC's determination is based on HED's evaluation of the Cheminova PWG report on the female Fischer 344 rat liver tumors. Also included are: revisions due to inconsistencies or errors identified in the CARC #1 report (Attachments 25 and 26); explanation of issues that needed further clarification; and references to minority opinions expressed by Dr. Brian Dementi (in this report and Attachments 1 - 22). Attachments 1 - 22 were written prior to the receipt and evaluation of the PWG report submitted by Cheminova regarding liver tumors in the female Fischer 344 rats.

For the 2-February-2000 report, Dr. Brian Dementi, Toxicology Branch 1, presented the experimental designs for the chronic bioassays including: survival data, body weight effects, cholinesterase data, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of the dose levels tested, and the weight of the evidence for the carcinogenicity of malathion and malaoxon. Dr. Dementi's memoranda regarding the assessment of the carcinogenicity of malathion and malaoxon that were forwarded to the Chairman/Executive Secretary of CARC are presented in Attachments 1 - 22. At the 12-April-2000 meeting, Dr. Dementi presented the results of the PWG (female Fischer 344 rat liver tumors) and Dr. Copley presented the other two issues (oral neoplasms and MCL).

Groups of male and female **B6C3F1 mice** received **malathion** in their diet at 0, 100, 800, 8000 or 16,000 ppm for 18 months. These dietary concentrations were equivalent to doses of 0, 17.4, 143, 1476 or 2978 mg/kg/day in males and 0, 20.8, 167, 1707 or 3448 mg/kg/day for females, respectively. The Committee concluded that in mice, the 800 ppm dose level was adequate to assess the carcinogenic potential of malathion, based on the statistically significant decrease in plasma and RBC cholinesterase activity (36% and 58%, respectively) in females and the biologically significant decrease (24% and 44%) in males. However, the 8000 and 16,000 ppm doses were excessive based on severe plasma (90 to 95%), severe red blood cell (RBC) (92 to 96%) and marked brain (20 to

43%) cholinesterase inhibition as well as decreased absolute body weight compared to controls (9.7 to 20%) in both sexes.

Groups of male and female **Fischer 344 rats** received **malathion** in their diet at concentrations of 0, 50, 500, 6000 or 12,000 ppm for 24 months; the low dose was initially 100 ppm, but was reduced to 50 ppm in both sexes from the 3 month time point for the duration of the study due to red blood cell cholinesterase inhibition among females at 100 ppm. These dietary concentrations were equivalent to doses of 0, 2, 29, 359 or 739 mg/kg/day in males and 0, 3, 35, 415 or 868 mg/kg/day for females, respectively. The Committee concluded that the 500 ppm dose in males was adequate to assess the carcinogenic potential of malathion based on a non-statistically, but biologically significant increase in mortality at this concentration (47% as compared to 33% in controls); and a decrease in plasma cholinesterase (29%, $p \leq 0.01$). The 6000 ppm dose in females was considered adequate based on a decrease in plasma, RBC and brain cholinesterase (61, 44 and 18 %, respectively). This dose was one-half the next dose where mortality was increased. However, 6000 ppm in males was considered excessive due to increased mortality (74%); and the 12,000 ppm dose was excessive in both sexes based on the severe inhibition of plasma (89%), red blood cell (52%) and brain (67%) cholinesterase activity in females and increased mortality in males (100%) and females (64%).

Groups of **Fischer 344 rats** were fed diets containing **malaoxon** at 0, 20, 1000 or 2000 ppm for 24 months. These dietary concentrations were equivalent to doses of 0, 1, 57 or 114 mg/kg/day in males and 0, 1, 68 or 141 mg/kg/day for females, respectively. The Committee concluded that the dose level of 1000 ppm, was adequate to assess the carcinogenic potential of malaoxon because it was one-half the dose (2000 ppm) causing excessive toxicity. The 2000 ppm dose was excessive due to increased mortality (53% in males and 49% in females) and severe inhibition of plasma (83-96%), red blood cell (54-66%) and brain (11-78%) cholinesterase activity.

The Committee concluded that there is evidence of carcinogenicity in both sexes of mice at the two highest dose levels of malathion tested which were considered excessive. There is no evidence of carcinogenicity in male or female mice at the lower doses. Evidence for carcinogenicity in mice is demonstrated by the presence of liver tumors in both sexes. **The Committee further concluded that there is evidence of carcinogenicity for malathion in female rats** at the highest dose although this dose was considered excessive. **The Committee determined that the oral (females at 6000 and 12,000 ppm) and nasal tumors (females at 6000 and 12,000 ppm and males at 12,000 ppm) could not be distinguished as either treatment-related or of random occurrence.**

Liver Tumors - Mice. In **male mice** (based on the Pathology Work Group Re-Read), there was a positive trend (p=0.000) for **liver** adenomas and the combined tumors (adenomas/carcinomas). The incidence of adenomas was significantly increased at 8000 ppm (14/55, 25%, p = 0.0103) and 16,000 ppm (49/51, 96%, p = 0.000) when compared to controls (4/54, 7%). Similarly, the combined tumors (adenomas/carcinomas) showed pair-wise significance at 8000 ppm (15/55, 27%, p=0.006) and 16,000 ppm (49/51, 96%, p=0.000) when compared to controls (4/54, 7%). Although carcinomas were seen at 100 ppm, 800 ppm and 8000 ppm compared to zero in the controls, none of the incidences showed statistical significance nor there was a dose-related increase at any dose level.

When compared with the historical control ranges: the incidences of adenomas at the 8000 ppm (25%) and 16,000 ppm (96%) doses exceeded the historical control range (14 to 22%). The incidences of carcinomas at 800 ppm (5%) and 8000 ppm (4%) doses were within the historical control range (0 - 6.4%). No carcinomas were seen at 16,000 ppm. The incidence of carcinomas at 100 ppm (7%) was slightly outside the historical control range and well above the mean value in a small historical control data base.

In **female mice**, there was a positive trend (p=0.000) for **liver** adenomas and the combined tumors (adenomas/carcinomas). The incidence of adenomas was significantly increased at 8000 ppm (9/52, 17%, p = 0.001) and 16,000 ppm (42/51, 82%, p = 0.000) when compared to controls (0/55). Similarly, the combined tumors (adenomas/carcinomas) showed pair-wise significance at 8000 ppm (10/52, 19%, p=0.003) and 16,000 ppm (43/51, 84%, p=0.000) when compared to controls (1/55, 2%). No statistically significant increases in carcinomas alone were seen at any dose.

Liver Tumors - Rats. There was no treatment related increase in liver tumors in **male rats**. In **female rats** (based on the Pathology Work Group Re-Read), there was a positive trend (p = 0.005) for adenomas. The incidence of adenomas was significantly increased by pair-wise comparison at 12,000 ppm (5/38, 13%, p = 0.009) when compared to controls (0/41). There were no carcinomas in any group.

When compared to the historical control data of the testing laboratory, the incidences of adenomas at 12,000 ppm (13%) exceeded the historical control range (0 to 5%) and mean (1.6%). In addition, the incidence of adenomas exceeded the historical control incidence (adenomas, 0.44%) of the NTP (1998 report).

The Committee concluded that although the incidence of liver tumors in female rats was observed only at an excessively toxic dose (12,000 ppm), it provided evidence of carcinogenicity because: 1) the incidence was statistically significant by pair-wise comparison; 2) there was a statistical trend; 3) the incidence was outside the range of both the testing facility and NTP historical control data bases.

Nasal Tumors - Rats. In **male rats**, there was an **adenoma of the olfactory epithelium** at 6000 ppm and an **adenoma of the respiratory epithelium** at 12,000 ppm compared to zero in the controls. In **female rats,** there was an **adenoma of the respiratory epithelium** at 6000 ppm and 12,000 ppm compared to zero in the controls.

The incidence of nasal adenomas of the respiratory epithelium in this study (1 in the 6000 ppm females and 1at 12,000 ppm in both sexes) exceeded the historical control incidence (0/240 males and 0/240 females). In addition, the NTP (1990 report, combined dietary and inhalation studies) reported respiratory tract tumors in the respiratory epithelium of 6/4000 male rats, in the olfactory epithelium of 0/4000 males and none of either type in females. Furthermore, four of these 6/4000 respiratory epithelial tumors were squamous cell tumors not adenomas of the respiratory epithelium. Therefore, the relevant historical control incidence for respiratory epithelial adenoma is only 2/4000 in males.

Of the four nasal tumors, one in each sex at the two highest dose levels, only one tumor in

the 6000 ppm dose in the female was at a dose that was not considered excessive. The biological significance of the adenoma of the olfactory epithelium (6000 ppm male) is unknown since it is from a different cell of origin and this type of tumor (esthesioneural epithelial neoplasm) should not be combined with other tumors of the respiratory nasal cavity. The Committee postulated that direct contact with malathion (by volatilization from the feed or by inhalation of the feed through the nose) was a possible explanation for the nasal tumors. However, there was no evidence to support or refute that the tumorigenicity was due to exposure by the inhalation or systemic route. To the contrary, the tumors occurred in section five, a section where there was little to no evidence of increased inflammation. Therefore, the Committee concluded that a systemic effect could not be unequivocally ruled out.

The Committee concluded that it could not determine whether nasal tumors were either treatment-related or due to random occurrence. On the one hand: (1) there was no dose response over a wide range of doses (100/50 to 12,000 ppm); (2) there was no statistical significance; (3) there were only adenomas, one in each of two doses for females and only one at the high dose in males; (4) the high dose in both male and females were considered excessively toxic; and (5) these tumors occurred in section 5 where there was little to no evidence of non-neoplastic lesions in the nasal mucosa. On the other hand: (1) an adenoma of the respiratory epithelium was seen in one female at 6000 ppm (not an excessive dose); (2) spontaneous nasal tumors are very rare in rats, there were no nasal tumors in the concurrent controls and the incidences exceeded the historical control incidence of the testing laboratory and NTP. The CARC also concluded that for males, the biological significance of the single olfactory epithelial tumor at 6000 ppm is unknown, since it is from a different cell of origin (esthesioneural epithelial neoplasm) and this type of tumor should not be combined with nasal respiratory epithelial neoplasms.

Oral Cavity Tumors - Rats. In **male rats**, there was one **squamous cell papilloma** of the palate at 100/50 ppm compared to zero in all other groups, including controls. In **female rats,** there was a **squamous cell carcinoma** of the alveolus of the tooth at 100/50 ppm, a **squamous cell papilloma** of the palate at 6000 ppm and a **squamous cell carcinoma** of the palate at 12,000 ppm compared to zero of all three tumor types in the controls. There is considerable uncertainty however, as to the actual incidence of these tumors and how many animals had this tissue examined since the oral mucosa was not considered a routine tissue for histologic examination.

The single occurrence of a low dose tumor in males was considered to be incidental background since there were no tumors at the higher doses, even with the large dose spread from 100/50 to 12,000 ppm. For females however, the incidence of oral squamous cell tumors in this study (1 at 6000 ppm and 1 at 12,000 ppm) exceeded the historical control incidence from inhalation studies at the testing facility (0/240 males and 0/240 females). In addition, the NTP (1998 report) reported: squamous cell papilloma - females 2/901 (0.22%), squamous cell carcinoma - females 0/901 (0%).

It was difficult to judge the significance of the low dose alveolar tumor since the oral cavity was not routinely examined in this study and the tumor was only seen in one low dose female. Of the two oral palate tumors, one at each of the two high doses, only the one adenoma in the 6000 ppm

female was at a dose that was not considered excessive.

The Committee concluded that it could not determine whether the oral cavity tumors in females were treatment-related or due to random occurrence. On the one hand: (1) there was no dose response over a wide range of doses (100/50 to 12,000 ppm); (2) there was no statistical significance; (3) the high dose in the females was considered excessively toxic. On the other hand: (1) a squamous cell papilloma of the palate was seen in one female at 6000 ppm (not an excessive dose); (2) spontaneous oral tumors are very rare in rats, there were no oral tumors in the concurrent controls and the incidences exceeded the historical control incidence of the testing laboratory and NTP; (3) due to the lack of systematic pathologic evaluation of the oral mucosa, there is uncertainty as to the actual incidence of oral tumors. However, the CARC determined that a recut would not alter their conclusion.

The Committee concluded that the following tumors are NOT treatment related:

Male rats - 1) **thyroid gland (follicular cell) -** there was neither statistical (other than a positive trend for combined adenomas and carcinomas) nor biological significance for any tumor type. Although there was no evidence that the above tumors are treatment related in rats at any dose level, the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12,000 ppm, where mortality was 74% and 100%, respectively. There is, however, no evidence to either support or refute this supposition.
2) **thyroid gland (C-cell) -** there was neither statistical (other that carcinomas in the 500 ppm group) nor biological significance, there was no dose-response relationship, and the combined tumor incidences in treated groups were comparable to those seen in the concurrent control group.
3) **testes (interstitial cell)** - tumor incidences of this nonfatal tumor were approaching 100% in all groups including controls, and positive statistical significance was considered to be an artifact in the Peto's Prevalence Analyses due to high mortality rather than biological significance.
4) **liver** - there was neither statistical nor biological significance and there was no dose-response relationship. Although there was no evidence that the above tumors were treatment related in rats at any dose level, the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12,000 ppm, where mortality was 74% and 100%, respectively. There is, however, no evidence to either support or refute this supposition.
5) **mononuclear cell leukemia** (MCL) - there was no indication of increased incidence or early onset, this tumor occurs commonly in Fischer 344 rats, the incidences were within historical control ranges, there was no statistical significance at any dose, and there was no dose response. Further more: (a) the CARC considered attributing the cause of death to MCL as subjective and not a reliable indicator of increased severity of this tumor; (b) using the incidence of deaths in leukemic animals caused by MCL as a measure of severity is not reliable because establishing a cause of death is subjective in older rats with possible multiple aging processes.

Female rats - 6) **pituitary gland (par distalis) -** the tumor incidences and types in treated groups were comparable to those seen in the concurrent control group; there was neither statistical nor biological significance; and there was no dose-response relationship.
7) **uterus (various types) -** the individual tumor incidences were low, the tumor incidences and types in treated groups were comparable to those seen in the concurrent control group; there was neither

statistical nor biological significance; and there was no dose-response relationship.

Results of the guideline genetic toxicology studies with malathion indicated that the test material did not cause gene mutations in bacteria or UDS in cultured rat hepatocytes. Similarly, malathion was neither clastogenic nor aneugenic up to doses that showed clear cytotoxicity for the target tissue *in vivo*. The CARC included that *in vitro* and *in vivo* findings from the open literature should be interpreted with caution since positive results were seen at cytotoxic doses and/or the types of induced aberrations were asymmetric and, therefore, not consistent with cell survival. The question of test material was also an issue. Although the structure of malathion suggests electrophilicity, **the Committee concluded that the weight of the evidence supports neither a mutagenic hazard nor a role for mutagenicity in the carcinogenicity associated with malathion.**

Malaoxon, the active cholinesterase inhibiting metabolite of malathion, was not carcinogenic in male or female rats when tested at doses that were judged to be adequate to assess its carcinogenic potential. MCL was not considered to be treatment related since: (1) statistical significance was seen only in males at a dose that was determined to be excessive, (2) there was no dose-response, and (3) the incidences were within the historical control range of the testing laboratory. Malaoxon was non-mutagenic in bacteria, was not clastogenic in cultured Chinese hamster ovary (CHO) cells, but did produce positive results without metabolic activation in the mouse lymphoma assay. Malaoxon caused sister chromatid exchanges in CHO cells in the absence of metabolic activation. Malaoxon has a structure similar to malathion; hence, the possibility of electrophilicity may also apply, despite the evidence of no carcinogenicity.

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (July 1999), the Committee at the 12-April-2000 meeting, classified malathion as **"suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential"** by all routes of exposure. This classification was based on the following factors:

(i) occurrence of liver tumors in male and female B6C3F1 mice and in female Fischer 344 rats only at excessive doses (statistically significant and outside historical control);

(ii) the presence of a few rare tumors, oral palate mucosa in females and nasal respiratory epithelium in male and female Fischer 344 rats. With the exception of one nasal and one oral tumor in female rats, all other tumor types were determined to occur at excessive doses or were unrelated to treatment with malathion. These tumors can not be distinguished as either treatment related or due to random occurrence;

(iii) the evidence for mutagenicity is not supportive of a mutagenic concern in carcinogenicity; and

(iv) malaoxon, a structurally related chemical, is not carcinogenic in male or female Fischer 344 rats.

The "suggestive" classification was supported by eleven out of sixteen CARC members present at the meeting. Four of the sixteen members of the CARC present at the meeting, thought that the evidence for malathion's cancer potential was weaker than a "suggestive" classification. There were two votes for, "data are inadequate for an assessment of human carcinogenic potential" and two votes for "not likely to be carcinogenic to humans." These opinions were based, in part, on the consideration that: 1) the increase in liver tumors was due to hepatocellular adenomas (benign

tumors); 2) there was no statistical significance at non-excessive doses (significance only in the presence of excessive toxicity); 3) the oral and nasal tumors were not considered treatment-related. In addition, they believed that the dose range for malathion's cancer effects was well defined and limited to excessive or near excessive doses. One member abstained.

Quantitative risk assessment for carcinogenicity is NOT required since the Committee classified malathion as having suggestive evidence for cancer. A cancer dose-response assessment, e.g. a low dose linear extrapolation model, is not indicated for pesticides in the "suggestive" category.

I. INTRODUCTION

On 24-September, 8-October, 15-October-1997, 10-June-1998, 24-February-1999 and 23-June-1999, the Health Effects Division's Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of malathion. The Committee reviewed the following studies: 1) Carcinogenicity study in B6C3F1 mice; 2) Combined chronic toxicity/carcinogenicity study in Fischer 344 rats with malathion; and 3) the Combined chronic toxicity/carcinogenicity study with malaoxon, the active cholinesterase inhibiting metabolite of malathion in F344 rats. Relevant subchronic, chronic and mutagenicity studies were also reviewed at these meetings, as well as the results of the studies conducted with malathion and/or malaoxon (during 1978-80) by the National Cancer Institute/National Toxicology Program (NCI/NTP). On 12-April-2000, the CARC met to evaluate: 1) a new Pathology Working Group (PWG) report on the female Fischer 344 rat liver tumors; 2) two issues raised by Dr. Dementi regarding the evaluation of malathion (items 4–mononuclear cell leukemia in Fischer 344 male rats and 7–oral tumors in Fischer 344 female rats from Attachment 25); 3) the 29-March-2000 letter from Jellinek, Schwartz & Connally, Inc. to Patricia Moe, Re: Comments on EPA's Risk Assessments for Malathion; 4) discuss the weight of evidence and cancer classification for malathion based on the previously listed information.

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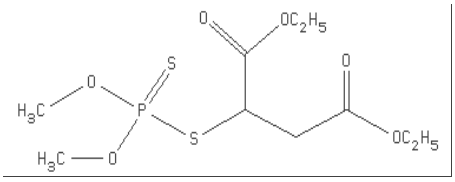
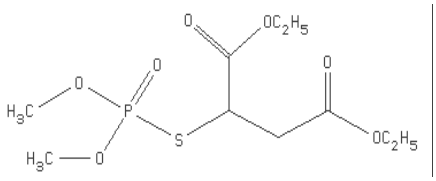
II. BACKGROUND INFORMATION



Malathion



Malaoxon

PC Code: 057701
CAS No. 121-75-5

In 1990, the malathion carcinogenicity data base was considered by the HED Cancer Peer Review Committee (CPRC). At that time, five NCI carcinogenicity studies plus a contract laboratory carcinogenicity study constituted the principal body of information on carcinogenicity under review by that committee. Specifically, the five NCI bioassays included studies of malathion in Osborne-Mendel rats, F344 rats and B6C3F1 mice, and of malaoxon in F344 rats and B6C3F1 mice. The contract laboratory study was a 2-year malathion study in Sprague-Dawley rats performed by Food and Drug Laboratories, Waverly, New York.

In 1990, the CPRC review of these six studies took into consideration the registrant's (Cheminova Inc.) assessment of the studies as well as an NTP reexamination of selected tissues in three of the NCI studies (malathion Osborne-Mendel and F344 rat studies and malaoxon F344 rat study). The suggested carcinogenic response of these studies included the following:

Species	Strain	Tumor Type/Sex
		Malathion
Mouse:	B6C3F1	Neoplastic nodules/hepatocellular carcinomas, males
Rat:	Sprague-Dawley	C-cell neoplasms of thyroid glands, female
		Mammary tumors and uterine polyps, female
Rat:	Fischer 344	Pheochromocytoma of adrenal gland, male
		Leukemia, male
Rat:	Osborne-Mendel	C-cell neoplasms of thyroid glands, male
		Follicular cell neoplasms of thyroid glands, both sexes
		Pheochromocytoma of adrenal gland, male
		Malaoxon
Mouse:	B6C3F1	No evidence of carcinogenicity
Rat:	Fischer 344	C-Cell neoplasms of thyroid glands, male and female
		Pheochrocytoma of the adrenal gland, male
		Mammary gland adenomas, female
		Lymphoma of hematopoietic, male

In 1990, the CPRC classified malathion as a Group D chemical, (not classifiable as to human

carcinogenicity) based on the inadequacy of the available studies to make definitive determinations on the carcinogenicity of malathion or malaoxon. The CPRC agreed with the NTP re-analysis that there was no clear evidence of carcinogenicity due to malathion or malaoxon administration in most of these studies. However, the Committee felt that there were many issues regarding the adequacy of each study which needed to be addressed before a firm conclusion regarding the carcinogenic potential of malathion could be made. In addition, while there may have been doubts about the significance of each tumor type in each of the individual studies, there was the suggestive appearance of similar tumors (e.g., C-cell tumors of thyroid gland and pheochromocytoma of adrenal gland) and of multiple tumors occurring in more than one study. There was also some evidence from mutagenicity studies suggesting that a genetic component for malathion and malaoxon was possible. These factors provided weight to the evidence of possible carcinogenic effects that could not be totally dismissed (Cancer Peer Review for Malathion dated 12-April-1990; HED Document No. 008386).

In 1990, the CPRC reaffirmed the recommendation of the 1988 Registration Standard for the Registrant to perform additional carcinogenicity studies with malathion and malaoxon. The 1988 Registration Standard had required a new malathion carcinogenicity study in B6C3F1 mice, a malaoxon chronic toxicity/carcinogenicity study in Fischer 344 rats, and a malathion chronic toxicity study in Fischer 344 rats at doses similar to, or higher than those in the NTP study (Cancer Peer Review for Malathion dated 12-April-1990; HED Document No. 008386). These studies were completed and reviewer for the 2-February-2000 CARC report. They constitute the principal body of information in the current evaluation of the carcinogenic potential of malathion and malaoxon.

The 2-February-2000 CARC report classified malathion as “likely to be carcinogenic to humans”. In that document, the Committee recommended that quantification of risk be estimated using the most potent unit risk, Q_1*, which was female rat liver adenoma plus carcinoma combined tumor rates at 1.52×10^{-3} in human equivalents.[1]

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Carcinogenicity Study with Malathion in B6C3F1 Mice

RW Slauter: "18-Month Oral (Dietary) Oncogenicity Study in Mice." 10/12/94. Study No. 668-001. Testing facility: International Research and Development Corporation (IRDC), Mattawan, MI (MRID No. 43407201).

A. Experimental Design

[1] See item # 14 in Attachment 25, memorandum titled, “Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion” dated March 30, 2000 for a minority opinion expressed by Dr. Brian Dementi and the CARC response. Additional details can be found in Attachments # 1 - 22, written by Dr. Dementi. However this issue was revisited by the CARC at the 12-April-2000 meeting.

Technical malathion (96.4% a.i.) was administered in the diet to groups of 65 male and 65 female B6C3F1 BR strain mice at dose levels of 0 (control) 100, 800, 8000 or 16,000 ppm (equivalent to 0, 17.4, 143, 1476 or 2978 mg/kg/day in males and to 0, 20.8, 167, 1707 or 3448 mg/kg/day in females). Ten mice/sex/group were sacrificed at 12 months and the remaining survivors were sacrificed at 18 months.

B. Discussion of Tumor Data

(i) Liver Tumors

The incidences of hepatocellular tumors were increased in both sexes of mice as shown in **Table 1**.

Male mice had significant increasing trends, and significant differences in pair-wise comparisons of the 8000 and 16,000 ppm dose groups with the controls, for liver adenomas, and adenomas/carcinomas combined, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 100 and 8000 ppm dose groups with the controls for liver carcinomas, both at $p < 0.05$. There was also a significant difference in the pair-wise comparison of the 100 ppm dose group with the controls for combined adenomas/carcinomas ($p < 0.01$).

Female mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 8000 and 16,000 ppm dose groups with the controls, for liver adenomas, and adenomas/carcinomas combined, all at $p < 0.01$.

The Committee concluded that the liver tissues/slides from male mice from all dose levels should be re-evaluated and submitted to a pathology working group (PWG) for peer review. This conclusion was based on: 1) the statistically significant increases in hepatocellular tumors in male mice at the low-(100 ppm), mid-high (8000 ppm) and high-(16,000 ppm) doses but not at the mid dose (800 ppm); and 2) the apparent low tumor incidence in the concurrent control (male) mice.

As requested by the Committee, a re-read of the male mouse liver pathology slides was conducted by a PWG and the results were submitted to the Agency. **The Committee accepted the results of the re-read of the male mouse liver tumors by the PWG.** The qualitative analysis of the re-read of the liver tumors is presented in **Table 2.**

There were significant differences in the pair-wise comparisons of the 16,000 ppm dose group with the controls, for liver adenomas, and combined adenomas/carcinomas, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 8000 ppm dose group with the controls for liver adenomas at $p < 0.05$, and combined liver adenomas /carcinomas at $p < 0.01$. There also were significant increasing trends for adenomas and combined adenomas/carcinomas ($p < 0.01$). Increased incidences of adenomas, carcinomas and combined adenomas/carcinomas were seen at 100 ppm and 800 ppm, but none of the increases showed either statistical significance or a dose-response relationship.

The Committee discussed the differences between the original diagnosis of the tumor incidences and those following the re-read by the PWG (**Table 3a**). The Committee also discussed the "multiplicity" component of the liver tumors in tumor-bearing animals (i.e., the presence of adenomas and carcinomas in the different lobes of the liver in the same mouse (**Table 3b)**. They observed a large increase in multiple adenomas only at the high dose. The significance of this finding is unclear since it occurs at an excessively toxic dose.

Table 1: Mice: Based on the Original 1994 Pathology Report - Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results.

Tumor Type	Sex	0 ppm	100 ppm	800 ppm	8000 ppm	16000 ppm
Adenomas	**Males**	1/54	6[a] /54	2/55	**13/55**	**49/51**
%		2	11	4	**24**	**96**
p=		**0.000****	0.056	0.507	**0.001****	**0.000****
%	**Females**	**0/55**	1/53	0/53	**9/52**	**42[b]/51**
p=		**0**	2	0	**17**	**82**
		0.000**	0.491	1.000	**0.001****	**0.000****
Carcinomas	**Males**	0/54	**6/54**	3[c] /55	**6/55**	1/51
%		0	**11**	5	**11**	2
p=		0.345	**0.014***	0.125	**0.014***	0.486
%	**Females**	1[d]/55	0/53	2/53	1/52	2/51
p=		2	0	4	2	4
		0.183	0.509	0.486	0.738	0.471
Combined	**Males**	1/54	**10[e] /54**	5/55	**18[f] /55**	**49[f]/51**
%		2	**19**	9	**33**	**96**
p=		**0.000****	**0.004****	0.107	**0.000****	**0.000****
%	**Females**	1/55	1/53	2/53	**10/52**	**43[g]/51**
p=		2	2	4	**19**	**84**
		0.000**	0.743	0.486	**0.003****	**0.000****

+= Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 53 interim sacrifice animals (Statistical Analysis - Brunsman, May 2, 1997)

[a] First liver adenoma in male observed at week 53, dose 16,000 ppm, in an interim sacrifice animal. Second liver adenoma in male seen at week 79, dose 100 ppm, in a terminal sacrifice animal.

[b] First liver adenoma in female observed at week 78, dose 16,000 ppm.

[c] First liver carcinoma in male observed at week 65, dose 800 ppm.

[d] First liver carcinoma in female observed at week 79, dose 0 ppm.

[e] Two males at 100 ppm had both an adenoma and a carcinoma.

[f] One male in each of the 8000 and 16,000 ppm dose groups had both an adenoma and a carcinoma.

[g] One female at 16,000 ppm dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. One male in the 16,000 ppm dose group of the interim sacrifice group had a liver adenoma. One female in the 16,000 ppm dose group which was accidentally killed had a liver adenoma.

Significance of trend denoted at control.

Significance of pair-wise comparisons with control denoted at dose level.

If *, then $p < 0.05$; If **, then $p < 0.01$

Table 2. Male Mice: PWG Re-read, 1998 - Liver Tumor Rates[+] and Exact Trend and Fisher's Exact Test Results.

Tumor Type	0 ppm	100 ppm	800 ppm	8000 ppm	16000 ppm
Adenomas	4/54	8[a] /54	7/55	**14[a] /55**	**49[a] /51**
%	7	15	13	**25**	**96**
p=	**0.000****	0.180	0.274	**0.0103***	**0.000****
Carcinomas	0/54	4/54	2[b] /55	2/55	0/51
%	0	7	5	4	0
p=	0.128	0.059	0.252	0.252	1.0
Combined	4/54	10[c] /54	9/55	**15[d] /55**	**49/51**
%	7	19	16	**27**	**96**
p==	**0.000****	0.075	0.125	**0.006****	**0.000****

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 53 interim sacrifice animals (Statistical Analysis, Brunsman, February, 16, 1999).

[a] First liver adenoma observed at week 53, dose 16,000 ppm, in an interim sacrifice animals. Subsequent liver adenomas observed at week 79, simultaneously in the 100, 8000 and 16,000 ppm dose groups, in terminal sacrifice animals.

[b] First liver carcinoma observed at week 65, dose 800 ppm

[c] Two animals in the 100 ppm dose group had both an adenoma and a carcinoma

[d] One animal in the 8000 ppm dose group had both an adenoma and a carcinoma

Note: Interim sacrifice animals are not included in this analysis. One male in the 16,000 ppm dose group of the interim sacrifice group had a liver adenoma.

Significance of trend denoted at control.

Significance of pair-wise comparisons with control denoted at dose level.

If *, then p <0.05; If **, then p <0.01

Table 3a. Male Mice: Summary of the Changes in Tumor Incidences Between the Original Diagnosis (i.e., the original pathology report, **Table 1**) and the re-evaluation (PWG report, **Table 2**)

	ORIGINAL DIAGNOSIS			REEVALUATION- PWG		
Dose(ppm)	**Adenoma**	**Carcinoma**	**Combined**	**Adenoma**	**Carcinoma**	**Combined**
Control	1	0	1	4	0	4
100	6	6	10	8	4	10
800	2	3	5	7	2	9
8,000	13	6	18	14	2	15
16,000	49	1	49	49	0	49

Table 3b. Male Mice: Incidences of "Single" and "Multiple" Tumors after Re-evaluation (PWG Re-read).

		0 ppm	100 ppm	800 ppm	8000 ppm	16,000 ppm
Adenomas-	Single	4	8	6	14	13
	Multiple	0	0	1	0	36
Carcinomas	Single	0	2	2	2	0
	Multiple	0	2	0	0	0
Adenoma/Carcinoma		0	2	0	1	0

Dr. Brennenke, the consulting pathologist, commented that in the evaluation of carcinogenicity, "tumor bearing animal" counts as one regardless of the number or multiplicity of any tumor type. Although carcinomas were observed in both sexes at all dose levels (except in males at 16,000 ppm and females at 100 ppm) the incidences showed neither a dose-response relationship nor statistical significance at any dose level. In addition, tumor incidences at the two high doses should be considered carefully since these dose levels were determined to be excessive for assessing carcinogenicity (Section D on Pages 9-10).

The incidences of liver tumors in male mice in this study (censored data[2]) were compared to the historical control data (non-censored) for male mice from five studies conducted at the testing laboratory (International Research and Development Corporation, Mattawan, MI). Based on the results of the PWG re-read, when compared with the historical control ranges: the incidences of adenomas at 8000 ppm (25%) and 16,000 ppm (96%) exceeded the historical control range (14 to 22%). For adenomas at the lower two doses of 100 (15%) and 800 ppm (13%), there was no statistical significance by pair-wise comparison, no dose related increase, and the values were within the historical control range of 14 to 22%.[3] The tumor response was actually at the low end of the range. The concurrent controls were well below the historical control range (7% as compared to 14 to 22%). This supported the conclusion that, what could have been interpreted as a treatment-related increase of tumors at the two low doses, was actually due to an unusually low control incidence. When compared to the historical control data, the incidence of carcinomas at 100 ppm (7%) was slightly outside the range (0 to 6%), and the incidences of carcinomas at 800 ppm (5%) and 8000 ppm (4%) were within the historical control range. In the 5 historical control studies, the incidences of liver carcinomas were: 0 in 3 studies; 1 mouse in one study (2.2%); and 3 mice in an another study (6.4%).

[2] Number of animals examined excluding those that died prior to the observance of the first tumor.

[3] Combined values and the means were not available.

The Committee concluded that there was evidence of carcinogenicity in both sexes of mice at the 8,000 and 16,000 ppm dose groups and there was no evidence of carcinogenicity in male or female mice at 100 or 800 ppm.[4]

In the 1978 NCI malathion study with B6C3F1 mice, liver tumors (11 carcinomas and 6 adenomas) were seen in 17 of 55 male mice at the highest dose tested (16,000 ppm); there was no carcinogenic response in female mice. Also in the NCI study, among females, the combined adenomas/carcinomas incidences were 2% at 0 ppm, 0% at 8000 ppm and 4% at 16,000 ppm in contrast to the present study where the tumor incidences in females were 2% at 0 ppm, 19% at 8000 ppm and 84% at 16,000 ppm. The Committee noted that there was no explanation as to why the tumor responses in the present study at the same dose levels were more pronounced than those seen in the NCI study.

(ii) Nasal Tumors (Mice)

At the 8-October-1997 meeting, the Committee elected to require histopathologic examination and peer review of microscopic slides of the nasal tissues from all animals in all dose groups in this carcinogenicity study in mice because of the concern for nasal tumors seen in the chronic toxicity/carcinogenicity study in rats (discussed later).

The tissue sections taken from five nasal regions in all mice were microscopically examined. This examination identified four neoplasms: a periodontal hemagiosarcoma in one control male; an odontoma in another control male; and an odontoma in each of two male mice in the low dose group.

The CARC concluded that the nasal neoplasms are not attributable to treatment since there was neither a statistical nor biological significance nor a dose-response relationship. Additionally, there is no evidence of a carcinogenic response in the nasal turbinate at any dose level.

C. Non-Neoplastic Lesions

Treatment-related non-neoplastic lesions of the liver manifested as hepatocellular hypertrophy were seen in both sexes of mice at the 8000 and 16,000 ppm dose levels. Incidence and severity of these lesions increased with dose. The incidences are summarized in **Table 4.**

[4] See item # 1 in Attachment 25, memorandum titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion" dated March 30, 2000 for a minority opinion expressed by Dr. Brian Dementi and the CARC response. Additional details can be found in Attachments # 1 - 22, written by Dr. Dementi.

Table 4. Non-Neoplastic Lesions of the Liver in Mice Fed Malathion for 18 Months.

Type of Lesion	0 ppm	100 ppm	800 ppm	8000 ppm	16000 ppm
MALES					
No. Examined	54	55	55	55	51
Hepatocellular Hypertrophy	0	0	0	55[a] (2.1)[b]	55 (3.1)
Mononuclear Cell Infiltration, Portal	0	2 (1.0)	0	4 (1.0)	4 (1.5)
Mononuclear Cell Foci, Parenchyma	5 (1.0)	4 (1.0)	7 (1.0)	9 (1.2)	4 (1.3)
Necrosis	2 (1.5)	2 (2.0)	1 (2.0)	3 (2.0)	5 (2.0)
FEMALES					
No. Examined	55	55	54	53	52
Hepatocellular Hypertrophy	0	0	0	53 (1.7)	52 (3.1)
Mononuclear Cell Infiltration, Portal	8 (1.0)	7 (1.3)	4 (1.0)	5 (1.0)	7 (1.0)
Mononuclear Cell Foci, Parenchyma	19 (1.0)	21 (1.0)	12 (1.1)	18 (1.1)	24 (1.0)
Necrosis	1 (1.0)	0	0	0	0

a = Incidences include mice that died, sacrificed in extremis, sacrificed at 18 month terminal sacrifice.
b = Indicate average severity score as follows: trace = 1.0; mild = 2.0, moderate = 3.0; severe = 4.0

Treatment-related non-neoplastic lesions of the nasal tissues were characterized as exudate, suppurative, increased glandular secretion, olfactory degeneration, olfactory atrophy and olfactory respiratory metaplasia in females at 800 ppm and in both sexes at 8000 and 16,000 ppm. The incidences are presented in **Table 5.**

Table 5. Non-Neoplastic Lesions of the Nasal Tissue in Mice[a].

Type of Lesion	No. Nasal Sections/ Animal	0 ppm	100 ppm	800 ppm	8000 ppm	16,000 ppm
MALES						
No. of Animals Examined		54	55	55	55	51
Exudate, Suppurative	5	3	7	5	31	35
Increased Glandular Secretion	5	0	5	4	116	108
Olfactory Degeneration	4	0	0	0	183	159
Olfactory Atrophy	3	0	0	0	58	46
Olfactory Respiratory Metaplasia	3	1	0	0	24	28
Hyperplasia of Bowman's Gland	4	0	0	0	59	54
FEMALES						
No. of Animals Examined		55	55	54	53	52
Exudate, Suppurative	5	0	2	12	30	43
Increased Glandular Secretion	4	7	6	89	149	133
Olfactory Degeneration	4	0	0	10	187	191
Olfactory Atrophy	3	1	3	48	71	67
Olfactory Respiratory Metaplasia	4	0	0	1	126	86
Hyperplasia of Bowman's Gland	3	0	0	0	0	32

a = Incidences presented are the <u>total of the lesions</u> observed in all sections of the nasal tissue.

D. <u>Adequacy of the Dosing for Assessment of Carcinogenicity</u>

There were no effects on survival rate in mice at any dose level for either sex. There were decreased absolute body weights at 8000 ppm and 16,000 ppm in both sexes, ranging from 14.3 to 20.0% in males and 9.7 to 16.1% in females, throughout the study. There were

no treatment-related clinical signs of toxicity at any dose level. The percent cholinesterase inhibition data are summarized in **Table 6.**

Statistically significant inhibition of plasma and RBC cholinesterase activity was observed in males at 8000 and 16,000 ppm and in females at 800, 8000, and 16,000 ppm; inhibition of brain cholinesterase activity was seen in males and females only at 16,000 ppm. At necropsy, liver "masses" were increased over controls in all male dose groups and at 16,000 ppm in females. Treatment-related non-neoplastic lesion, hypertrophy of the liver, was observed in both sexes of mice at 8000 and 16,000 ppm with incidence and severity of the lesion increasing with dose.

Table 6. Cholinesterase Activity in Mice Fed Malathion For 18 Months.

Percent Inhibition of Cholinesterase Activity At 18- Months						
Dose/Sex	**Plasma**		**Red blood cell**		**Brain**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
800 ppm	24	**36***	44	**58***	7	3
8000 ppm	**90****	**92****	**90****	**92****	23	20
16,000 ppm	**95****	**96****	**92****	**92****	**37****	**43****

If *, = $p \leq 0.05$; If, ** = $p \leq 0.01$

The Committee concluded that, based on the severity of cholinesterase inhibition in both sexes, the two top dose levels (8000 and 16,000 ppm) were excessive and that the 800 ppm dose was adequate to assess the carcinogenic potential of malathion in this strain of mice. The 800 ppm dose was determined to be adequate based on the statistically significant decrease in plasma and RBC cholinesterase activity (36% and 58%, respectively) in females and biologically significant decrease (24% and 44%) in males. The Committee noted that the degree of cholinesterase inhibition was less severe when compared to 8000 ppm and 16,000 ppm dose levels. The Committee further noted that the 8000 ppm (1476 mg/kg/day dose in males and 1707 mg/kg/day in females) dose was higher than the Limit Dose (1000 mg/kg/day) and the 16,000 ppm (2978 mg/kg/day in males and 3448 mg/kg/day in females) dose was more than twice the Limit Dose for carcinogenicity studies.

The two highest dose levels tested in this study (8000 and 16,000 ppm) were required by the Agency (Data Call-In, 15-June-1992) since they duplicated the levels tested in the 1978 NCI study in this strain of mice. In the 1978 NCI study, increased incidences of liver tumors in male mice were reported at 16,000 ppm, however, due to the equivocal nature of the findings, a clear association between liver tumors and malathion administration could not be established. In addition, study design flaws, uncertainties about the conduct of the study, and

lack of sufficient detail to allow independent statistical analysis of the data further compromised the usefulness of the NCI study. Therefore, the Agency required a new study to be performed under similar conditions in order to resolve the question of possible carcinogenicity of malathion in B6C3F1 mice.

2. Combined Chronic Toxicity/Carcinogenicity Study with Malathion in Fischer 344 Rats

Reference: Daly, W.I.: "A 24-Month Oral Toxicity/Oncogenicity Study of Malathion in the Rat via Dietary Administration," February 27, 1996. Lab. Study No.: 90-3641. Testing Facility: Huntington Life Sciences. East Milestone, NJ. (MRID Number: 43942901).

A. Experimental Design

Malathion Technical (97% a.i.) was administered in the diet to groups of (90/sex/dose) F344 rats at 0, 100/50, 500, 6000 or 12,000 ppm [equivalent to respective mean values of 0, 2, 29, 359 and 739 mg/kg/day (males) and 0, 3, 35, 415 and 868 mg/kg/day (females)] for two years. Ten rats/sex/group were sacrificed at 3 month and 6 month time intervals, primarily for ocular tissue assessments. A full 12 month interim sacrifice (not limited to ocular tissues) was performed on 15 animals. There were 55 rats/sex/group devoted to the full 2-year study. The low dose in the study was initially 100 ppm, but was reduced to 50 ppm in both sexes from the 3 month time point for the duration of the study due to the finding of statistically significant RBC cholinesterase inhibition in females.[5]

B. Discussion of Tumor Data

(i) Liver Tumors

Males - There was no evidence of treatment related increases or statistical significance in hepatocellular tumors (either adenomas or carcinomas) at any dose level in male rats. The incidence (uncensored) of adenomas for controls to the high dose is: 2/55 (3.6%), 2/55 (3.6%), 3/55 (5.5%), 2/55 (3.6%), and 1/55 (1.8%). The incidence (uncensored) of carcinomas for controls to the high dose is: 1/55 (1.8%), 2/55 (3.6%), 1/55 (1.8%), 1/55 (1.8%), and 0/55. It should be noted that there was excessive toxicity at the two high doses.

It was also noted that there was no evidence of liver carcinogenicity in male rats at any dose level, but the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12,000 ppm, where mortality was 74% and 100%, respectively as compared to 33% in controls. There is, however, no evidence to either support or refute this supposition.

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[5] See item # 13 in Attachment 25, memorandum titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion" dated March 30, 2000 for a minority opinion expressed by Dr. Brian Dementi and the CARC response. Additional details can be found in Attachments # 1 - 22, written by Dr. Dementi.

Females - Before the PWG re-read (see **Table 7a**)**,** there were pair-wise significant increases relative to the control group for adenomas and combined liver adenomas/carcinomas at 6000 ppm ($p < 0.05$) and 12,000 ppm ($p < 0.01$) in female rats. There were no statistically significant increases in hepatocellular carcinomas at any dose level in female rats. There were significant increasing trends for adenomas and combined adenomas/carcinomas ($p < 0.01$).

On 14 and 15-March-2000, Cheminova, Inc. conducted a re-evaluation of the female liver slides. This included a pathology peer review of all liver slides, followed by a PWG evaluation of all liver slides that contained the following: 1) any diagnoses by either the original study pathologist or the peer review pathologist of (a) cellular alteration (moderate and severe), (b) hyperplasia, (c) adenoma, (d) carcinoma; 2) any gross observations at necropsy. The CARC concluded that the PWG was conducted in accordance with FR Notice 94-5 and the new values for liver tumors presented in **Table 7b** should be used. **Table 7c** presents the comparison of diagnoses among the original study pathologist, peer review pathologist and the PWG consensus. **There was discussion regarding the occurrence of cellular alteration. However, it was determined that: 1) cellular alteration is not a reliable indicator of progression to neoplasia, and 2) there was no basis for considering this to be a preneoplastic lesion in this study since there was no increase of basophilic foci (based on the original study report).**

There were no carcinomas observed by the PWG in any group. For adenomas, there was a positive trend ($p > 0.01$) and pair-wise comparison at 12,000 ppm ($p > 0.01$). The incidence of liver adenomas at the 12,000 ppm dose in this study (censored data) were compared to the historical control data (non-censored) from studies conducted at the testing laboratory. The incidence of adenomas at 12,000 ppm (13%) exceeded the historical control range (0 to 5%) and mean (1.6%). In addition, this incidence exceeded the historical control incidences of the NTP, 1998 report for adenomas (4/901, 0.44%).

The Committee concluded that although the incidence of liver tumors in female rats was observed only at an excessively toxic dose (12,000 ppm), it provided evidence of carcinogenicity because: 1) the incidence was statistically significant by pair-wise comparison; 2) there was a statistical trend; 3) the incidence was outside the range of both the testing facility and NTP historical control data bases. It was also observed that this increase only occurred at an excessively toxic dose.

Table 7a. Female Rat: Liver Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas	0/40	1[a] /48	1/43	**3/39**	**3/29**
%	0	2	2	**8**	**10**
p=	**0.007****	0.240	0.168	**0.032***	**0.008****
Carcinomas	0/41	1/50	1/44	0/41	3[b] /38
%	0	2	2	0	8
p=	0.063	0.168	0.168	-	0.085
Combined	0/41	2/50	2/44	**3/41**	**6/38**
%	0	4	5	**7**	**16**
p==	**0.002****	0.134	0.085	**0.032***	**0.003****

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, July 16, 1997).
[a] First liver adenoma observed at week 103, dose 100/50 ppm.
[b] First liver carcinoma observed at week 101, dose 12,000 ppm
Note: Interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then p <0.05; If **, then p <0.01

Table 7b. Female Rats: PWG Re-read, 2000 - Liver Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas	0/41	1/50	2/44	0/41	5/38[a]
%	0	2	5	0	13
p=	0.005**	0.168	0.085	–	0.009**

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Burnam, April 25, 2000).
[a] First liver adenoma observed at week 101, dose 12,000 ppm.
Note: Interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then p <0.05; If **, then p <0.01

Table 7c. Female Rats: Summary of the Changes in Tumor Incidences Between the Original Diagnosis, Peer Review Pathologist and the PWG Consensus.

	Original Diagnosis			Peer Review Pathologist			PWG
Dose(ppm)	Adenoma	Carcinoma	Combined	Adenoma	Carcinoma	Combined	Adenoma
Control	0	0	0	0	0	0	0
100/50	1	1	2	1	1	2	1
500	1	1	2	1	1	2	2
6,000	3	0	3	2	0	2	0
12,000	3	3	6	4	2	6	5[a]

[a] Animal # 5512 had two adenomas

(ii). Nasal Tumors (Rat)

At the 24-September-1997 meeting, the Committee determined that nasal tissues had not been fully evaluated histopathologically in the original submission. Therefore, the Committee elected to require the histopathologic examination and peer review of microscopic slides of nasal tissues among rats of both sexes. The nasal/oral tissue sections taken from five nasal regions from all rats underwent microscopic examination. This was a nasal tissue reevaluation, and oral tissue findings (squamous cell tumors of the oral palate and alveolus of the tooth) were incidental in the nasal tissue assessment, which reflects only a partial histopathologic assessment of oral cavity tissues. At the 12-April-2000 meeting, the CARC reevaluated this lesion based on comments by Cheminova (letter dated 29-March-2000) and recommendations in the 30-March-2000 memorandum by Marion Copley titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion." The nasal/oral tumor incidences are presented in **Table 8**.

Table 8. Neoplastic Findings of the Nasal & Oral Tissues in Rats

TUMOR TYPE	Dose (ppm)				
	0	**100/50**	**500**	**6000**	**12000**
MALES (No. Examined for Nasal Only: 55/dose)[a]					
Nasal Olfactory Epithelium **Adenoma**	0	0	0	**1**	0
Nasal Respiratory Epithelium **Adenoma**	0	0	0	0	**1**
Oral Palate, Squamous Cell papilloma	0	1	0	0	0
FEMALES (No. Examined for Nasal Only: 55/dose)[a]					
Nasal Respiratory Epithelium **Adenoma**	0	0	0	**1**	**1**
Oral Tooth, Alveolus, Squamous Cell Carcinoma	0	1	0	0	0
Oral Palate, Squamous Cell Papilloma	0	0	0	1	0
Oral Palate, Squamous Cell Carcinoma	0	0	0	0	1

[a] Un-censored data; For nasal tumors this does not include the 3, 6 or 12 month interim sacrifice animals. There were no tumors in the intermediate sacrifice animals.
For oral tumors - the number of animals examined can not be determined. Oral tissue was only examined incidentally in some nasal tissue slides.

The Committee compared these nasal tumors to the historical control data from the testing laboratory as well as to the tumors of the "respiratory tract" seen in studies conducted at NTP. The incidence of nasal respiratory epithelial adenomas in this study (1 at 6000 ppm in females and 1 at 12,000 ppm in both sexes) exceeded the testing facility historical control incidence (0/240 males and 0/240 females). In male rats, there was also an adenoma of the olfactory epithelium at 6000 ppm. In addition, the NTP (1990 report, combined dietary and inhalation studies) reported respiratory tract tumors in the respiratory epithelium of 6/4000 male rats, in the olfactory epithelium of 0/4000 males, and none of either type in females. Furthermore, four of these 6/4000 respiratory epithelial tumors were squamous cell tumors, not adenomas of the respiratory epithelium. Thus, the relevant NTP historical control incidence for the tumor type in question is only 2/4000 males. The biological significance of the adenoma of the olfactory epithelium (6000 ppm male) is unknown since it is from a different cell of origin and this type of tumor (esthesioneural epithelial neoplasm) should not be combined with other tumors of the respiratory nasal cavity.

The Committee postulated that direct contact with malathion (by volatilization from the feed or by inhalation of the feed through the nose) was a possible explanation for the nasal tumors. However, there was no evidence to support or refute that the tumorigenicity was due to exposure by the inhalation or systemic route. Therefore, the Committee concluded that a systemic effect could not be unequivocally ruled out. The Committee noted that the Hazard Identification Assessment Review Committee (HIARC) determined that a new subchronic inhalation toxicity study in rats is required based on the results of the two-week range-finding study (MRID No. 44554301) and the lack of a NOAEL for cholinesterase inhibition and non-neoplastic lesions in the 90-day study (MRID No.43266601) (HIARC Report dated 12/22/98; HED Document No. 013032).[6]

The Committee concluded that it could not determine whether nasal tumors were either treatment-related or due to random occurrence. On the one hand: (1) there was no dose response over a wide range of doses (100/50 to 12,000 ppm); (2) there was no statistical significance; (3) there were only adenomas, one in each of two doses for females and only one at the high dose in males; (4) the high dose in both male and females were considered excessively toxic; and (5) these tumors occurred in section 5 where there was little to no evidence of non-neoplastic lesions in the nasal mucosa. On the other hand: (1) an adenoma of the respiratory epithelium was seen in one female at 6000 ppm (not an excessive dose); (2) spontaneous nasal tumors are very rare in rats, there were no nasal tumors in the concurrent controls and the incidences exceeded the historical control incidence of the testing laboratory and NTP. The CARC also concluded that for males, the biological significance of the single olfactory epithelial tumor at 6000 ppm is unknown, since it is from a different cell of origin (esthesioneural epithelial neoplasm) and this type of tumor should not be combined with nasal respiratory epithelial neoplasms.

(iii). Oral (Palate & Alveolar-tooth) Tumors (Rat)

At the 12-April-2000 meeting, the CARC reevaluated this lesion based on comments by Dr. Brian Dementi concerning historical control data and recommendations in the 30-March-2000 memorandum by Marion Copley titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion."

As noted in section (ii) Nasal Tumors (Rat), oral tissue findings (squamous cell tumors of the oral palate and alveolus of the tooth) were incidental in the nasal tissue assessment, which reflects only a partial histopathologic assessment of oral cavity tissues. As presented in **Table 8** above, oral palate tumors were observed in one 100/50 ppm male (squamous cell papilloma), one 6000 ppm female (squamous cell papilloma) and one 12,000 ppm female (squamous cell carcinoma). There was also a squamous cell papilloma of the alveolus of the tooth in one 100/50 ppm females. There were no oral tumors reported in the controls.

[6] See item # 6 in Attachment 25, memorandum titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion" dated March 30, 2000 for a minority opinion expressed by Dr. Brian Dementi and the CARC response. Additional details can be found in Attachments # 1 - 22, written by Dr. Dementi. This issue was revisited at the 12-April-2000 CARC meeting.

There was considerable uncertainty however, as to the actual incidence of these tumors and how many animals had this tissue examined since the oral mucosa was not considered a routine tissue for histologic examination. There was some discussion as to whether the oral tissues should be reexamined histologically in order to eliminate this uncertainty. However, a vote of the CARC members present at the meeting (taken by Notes mail after the meeting) resulted in an overwhelming response to the negative. It should be noted that the pathologist mentioned at the meeting that the ventral border of histology sections with the nasal mucosa usually contains hard or soft palate. However, since this tissue was not considered part of the routine protocol for this study, there was no mention of oral observations, other than the tumors noted in **Table 8.** It is possible that oral tissue was examined and found negative but results were not recorded because examination was not part of the protocol.

The single occurrence of a low dose tumor in males was considered to be incidental background since there were no tumors at the higher doses, even with the large dose spread from 100/50 to 12,000 ppm. For females however, the incidence of oral squamous cell tumors in this study (1 at 6000 ppm and 1 at 12,000 ppm) exceeded the historical control incidence from inhalation studies at the testing facility (0/240 males and 0/240 females). In addition, the NTP (1998 report) reported: squamous cell papilloma - females 2/901 (0.22%), squamous cell carcinoma - females 0/901 (0%).

It was difficult to determine the significance of the low dose alveolar tumor since the oral cavity was not routinely examined in this study and the tumor was only seen in one low dose female. Of the two oral palate tumors, one at each of the two high doses, only the one adenoma in the 6000 ppm female was at a dose that was not considered excessive.

The Committee concluded that it could not determine whether the oral cavity tumors in females were treatment-related or due to random occurrence. On the one hand: (1) there was no dose response over a wide range of doses (100/50 to 12,000 ppm); (2) there was no statistical significance; (3) the high dose in the females was considered excessively toxic. On the other hand: (1) a squamous cell papilloma of the palate was seen in one female at 6000 ppm (not an excessive dose); (2) spontaneous oral tumors are very rare in rats, there were no oral tumors in the concurrent controls and the incidences exceeded the historical control incidence of the testing laboratory and NTP; (3) due to the lack of systematic pathologic evaluation of the oral mucosa, there is uncertainty as to the actual incidence of oral tumors.[7] However, the CARC concluded that additional pathological evaluation would not alter their conclusion.

(iv). <u>Thyroid Follicular Cell Tumors</u> (Rat)

[7] See item # 7 in Attachment 25, memorandum titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion" dated March 30, 2000 for a minority opinion expressed by Dr. Brian Dementi and the CARC response. Additional details can be found in Attachments # 1 - 22, written by Dr. Dementi. This issue was revisited at the 12-April-2000 CARC meeting.

Historical control data (uncensored) from the testing facility indicate that in 6 studies with about 50 rats each (3 dietary and 3 inhalation), the mean for adenomas was about 1.3% (range 0 - 2%) and the mean for carcinomas was 1.7% (0 - 4). Historical control data from the NTP 1998 report indicate the mean for adenomas was 12.3% (2 - 24) and the mean for carcinomas was about 1.1% (range 0 - 4%).

Thyroid gland follicular cell tumors in male rats are presented in **Table 9.**

Table 9. Male Rat: Thyroid Follicular Cell Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas	2/55	1/54	1/51	4/51	4[a]/43
(%)	4	2	2	8	9
p=	0.063	-	-	0.150	0.378
Carcinomas	0/42	0/45	2/41	2[b] /26	0/0
(%)	0	0	5	8	0
p=	0.196	-	0.085	0.162	-
Combined	2/55	1/54	3/51	6/51	4/43
(%)	4	2	6	12	9
p=	**0.035**[*]	-	0.321	0.077	0.160

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, July 16, 1997).
[a] First thyroid follicular cell adenoma observed at week 76, dose 12,000 ppm.
[b] First thyroid follicular cell carcinoma observed at week 100, dose 6,000 ppm
Note: Interim sacrifice and accidental death animals are not included in this analysis. There were no thyroid follicular cell tumors in any of the interim sacrifice or accidental death animals.
Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then p <0.05; If **, then p <0.01

The Committee concluded that the thyroid follicular cell tumors are NOT treatment-related since there is neither a pair-wise significance nor a dose-response relationship for any tumor type (i.e., adenomas, carcinomas or combined adenomas/carcinomas); only a trend was seen for the combined tumors.[8] Additionally, there was no evidence of malathion induced thyroid toxicity in the database and there were no

[8] See item # 3 in Attachment 25, memorandum titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion" dated March 30, 2000 for a minority opinion expressed by Dr. Brian Dementi and the CARC response. Additional details can be found in Attachments # 1 - 22, written by Dr. Dementi.

supportive pre (non) neoplastic lesions in the thyroid glands of male or female rats.

(v). Thyroid C-Cell Tumors (Rat)

At the 8-October-1997 meeting, the Committee requested additional statistical analysis (Peto's Prevalence Test) of the thyroid C-cell tumor incidences in male rats as well as historical control data from the testing laboratory. Tumor incidences and results of the statistical analysis are presented in **Table 10a** (for all dose groups) and in **Table 10b** (excluding the top two doses)**.**

Table 10a. Male Rat: Thyroid C-Cell Tumor Rates[+] and Peto's Prevalence Test Results Including All Dose Groups

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas p=	13/53 (25%) 0.326	14/54 (26%) 0.461	10/50 (20%) -	6/50 (12%) -	4[a]/35 (11%) 0.242
Carcinomas p=	1/51 (2%) 0.556	2/50 (4%) 0.310	6[b]/45(13%) **0.012**[*]	2/43 (5%) 0.178	0/9 (0%) -
Combined p=	14/53 (25%) 0.430	16/54 (30%) 0.389	14[c]/50(28%) 0.403	8/50 (16%) -	4/35 (11%) 0.242

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, May 03, 1999).

[a] First thyroid C-cell adenoma observed at week 81, dose 12,000 ppm.

[b] First thyroid C-cell carcinoma observed at week 90, dose 500 ppm.

[c] Two animals in the 500 ppm had both an adenoma and a carcinoma.

Table 10b. Male Rat: Thyroid C-Cell Tumor Rates[+] and Peto's Prevalence Test Results Excluding Top Two Dose (6000 & 12,000 ppm) Groups

Tumor Type	0 ppm	100/50 ppm	500 ppm
Adenomas p=	13[a]/46 (28%) 0.737	14/47 (30%) 0.461	10/44 (23%) -
Carcinomas p=	1/51 (2%) **0.006****	2/50 (4%) 0.310	6[b] /45 (13%) **0.013***
Combined p=	14/51 (27%) 0.356	16/50 (32%) 0.394	14[c] /50 (31%) 0.332

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, May 03, 1999).
[a] First thyroid C-cell adenoma observed at week 97, dose 0 ppm.
[b] First thyroid C-cell carcinoma observed at week 90, dose 500 ppm.
[c] Two animals in the 500 ppm had both an adenoma and a carcinoma.
Note: Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then p <0.05; If **, then p <0.01

The Committee noted when all dose groups were included (**Table 10a**), male rats had a statistically significant (p=0.012) difference in the pair-wise comparison of the 500 ppm dose group with the controls for thyroid C-cell carcinomas. There were no other statistically significant differences in the pair-wise comparisons of the dosed groups with the controls, or any significant trends for C-cell adenomas, carcinomas or combined adenomas/carcinomas. Additionally, there was no dose-related increase for any tumor type. The Committee also observed that when the top two doses (6000 ppm and 12,000 ppm) were excluded (**Table 10b**) from the analysis there was a dose-related increase (2%, 4% and 13% at 0 ppm, 50 ppm and 500 ppm, respectively), a pair-wise significance (p=0.013) at 500 ppm, and the incidences at both doses exceeded the mean historical control incidence (6/239; 2.5%) for carcinomas in male rats.

Historical control data (uncensored) from the testing facility indicate that in 6 studies with about 50 rats each (3 dietary and 3 inhalation), the mean for adenomas was 21% (9 - 31) and the mean for carcinomas was about 2.5% (range 0 - 8%). Historical control data from the NTP 1998 report indicate the mean for adenomas was 12.3% (2 - 24%), for carcinomas was about 1.1% (range 0 - 4%) and for combined adenomas/carcinomas was 13.4% (4-24%).

There is statistical significance by pair-wise comparison (p<0.01) for thyroid C-cell carcinomas at the 500 ppm (both with and with out considering the 2 high doses) (2%, 4%, 13%, 5%, 0%, for controls to high dose). The CARC did consider the possibility that the excessive mortality in males at the top doses (74% at 6000 ppm and 100% at 12,000 ppm)

may have compromised the expression of this tumor at these (higher) doses. However this was discounted because at 6000 ppm, there were still 43 rats considered to be at risk (alive after the first occurrence of carcinoma) which was considered to be an adequate number for evaluation. Therefore, the CARC considered that there was no dose response in males and the increase at 500 ppm was due to variability rather than to malathion.

The incidences of combined thyroid C-cell tumors were determined to be the most appropriate tumor values for the final evaluation.[9] There were no other statistically significant differences in the pair-wise comparisons of the dosed groups with the controls nor any significant trends for C-cell adenomas or combined adenomas/carcinomas. Additionally, there was no evidence of malathion induced thyroid toxicity in the database and there were no supportive pre (non) neoplastic lesions in the thyroid glands of male or female rats.

The Committee concluded that the thyroid C-cell tumors are NOT attributable to treatment based on the combined tumor (adenomas/carcinomas) incidences. The combined tumors were determined to be the most appropriate tumor type for evaluation. For the combined tumors, there was no statistically significant trend, pair-wise significance, or dose-response at any dose level when all dose groups were included or when the top two doses were excluded from the analyses. Additionally, there was no evidence of malathion induced thyroid toxicity in the database and there were no supportive pre (non) neoplastic lesions in the thyroid glands of male or female rats.[10]

(vi). <u>Pituitary Tumors</u> (Rat)

At the 15-October-1997 meeting, the Committee noted that not all female pituitary glands had been examined microscopically, therefore, histopathologic examination and peer review of microscopic slides of pituitary glands from all female rats were required. The pituitary gland tumors (original results) observed in female rats are presented in **Table 11.**

[9] Also see Reference: McConnell, E. E., Solleveld, H. A., Swenberg, J. A. and Boorman, G. A. (1986) Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies. JNCI, 76, pp. 283-289.

[10] See item # 2 in Attachment 25, memorandum titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion" dated March 30, 2000 for a minority opinion expressed by Dr. Brian Dementi and the CARC response. Additional details can be found in Attachments # 1 - 22, written by Dr. Dementi.

Table 11. Female Rat: Pituitary Pars Distalis Tumor Rates[+] and Peto's Prevalence Test Results (Original Study Report).

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas	25/51	13/31	20[a]/34	17/33	14/53
(%)	49	42	59	52	26
p=	0.980	-	0.133	0.266	-
Carcinomas	0/50	1/30	3[b]/32	4/32	1/49
(%)	0	3	9	12	2
p=	0.778	0.319	**0.029***	**0.027***	0.369
Combined	25/51	14/31	23/34	21/33	15/53
(%)	49	45	68	64	28
p=	0.987	-	**0.033***	0.097	-

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, July 16, 1997).
[a] First pituitary pars distalis adenoma observed at week 56, dose 500 ppm.
[b] First pituitary pars distalis carcinoma observed at week 79, dose 500 ppm
Note: Interim sacrifice and accidental death animals are not included in this analysis. There were no pituitary pars distalis tumors in any of the interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then p <0.05; If **, then p <0.01

The histopathology examination of pituitary glands from all females (re-read) were completed and the results are presented in **Table 12.**

Table 12. Female Rat: Histopathology of the Pituitary Glands from ALL Animals (Re-Read).

Tumor Type	0 ppm		100/50 ppm		500 ppm		6000 ppm		12000 ppm	
No. Examined	88	87	90	90	87	89	87	88	88	89
Sex	M	F	M	F	M	F	M	F	M	F
Adenomas	20	25	23	23	16	27	17	18	5	17
Carcinomas	0	0	0	1	0	2	0	1	0	1

The Committee concluded that the pituitary tumors are NOT attributable to treatment since the incidences and types of tumors (adenoma and carcinoma) observed in the treated groups were comparable to those seen in the control group and since there was neither statistical significance nor dose-response for either pituitary tumor type.

(vii). Uterine Tumors

At the 15-October-1997 meeting, the Committee noted the presence of some rare/unusual uterine tumors which are shown in **Table 13.** Individually, the incidences of the uterine tumors were low. However, collectively the incidences of the uterine tumors were of a concern to the Committee. It was also noted that not all animals at the low, mid and mid-high doses were examined. Therefore, the Committee requested histopathologic examination and peer review of microscopic slides of the uteri from all females. The re-read histopathology of the uterine tumors from all animals is presented in **Table 14.**

Table 13. Female Rats: Incidence of Uterine Tumors (Original Pathology Report).

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
No. Examined	70	26	24	31	70
Deciduoma	0	1	0	0	0
Hemangioma	0	0	0	0	1
Endometrial Carcinoma	1	2	0	0	2
Endometrial, Carcinosarcoma	0	0	0	0	1
Stromal Sarcoma	0	1	0	0	0
Fibrosarcoma	0	1	0	0	0
Leiomyosarcoma	0	0	0	1	0

Table 14. Female Rat: Incidence of Uterine Tumors (Re-Read of ALL Animals).

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
No. Examined	90	90	90	90	90
Deciduoma	0	0	0	0	0
Hemangioma	0	0	0	0	1
Endometrial Adenoma	0	0	1	0	0
Endometrial Carcinoma	1	2	1	0	2
Endometrial Stromal Polyp	20	15	17	11	11
Fibrosarcoma	0	1	0	0	0
Fibroma	0	0	0	1	0
Leiomyoma	0	0	0	1	0
Leiomyosarcoma	0	0	0	1	0
Stromal Sarcoma	0	1	0	0	0

The Committee concluded that the uterine tumors are NOT treatment related since the incidences and types of tumors observed in the treated groups were comparable to those seen in the control animals and since there was neither statistical significance nor dose-response for any tumor type.

(viii). Testicular Tumors (Rat)

At the October 8, 1997 meeting the Committee evaluated the interstitial cell tumors of the testes presented in **Table 15**.

Table 15. Male Rat: Testes Interstitial Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

Tumor Type	0 ppm	100/ 50 ppm	500 ppm	6000 ppm	12000 ppm
Interstitial cell tumor	52/55	52/55	53/55	52/53	53 [a] /54
(%)	95	95	96	98	98
p=	**0.000****	-	**0.037***	**0.032***	**0.004****

[+] = Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, 16-July-1997).

[a] First testicular tumor observed at week 54, dose 0 ppm, in a 54-week interim sacrifice animal. First testicular tumor not in an interim sacrifice or accidental death animal observed at week 64, dose 12,000 ppm

Note: Interim sacrifice and accidental death animals are not included in this analysis. Two animals in the 0 ppm dose group and five animals in the 12,000 ppm dose group of the 54-week interim sacrifice group had this tumor. Two accidental death animals in the 6000 ppm dose group had this tumor.

Significance of trend denoted at control.

Significance of pair-wise comparisons with control denoted at dose level.

If *, then $p < 0.05$; If **, then $p < 0.01$

Male rats had a significant increasing trend, and a significant difference in the pair-wise comparison of the 12,000 ppm dose group with the controls for the interstitial cell tumor, both at $p < 0.01$—using the Peto's Prevalence Analyses protocol. There were also significant differences in the pair-wise comparisons of the 500 ppm and 6000 ppm dose groups with the controls for this tumor type, both at $p < 0.05$. Statistical analyses of this tumor in the study report concluded that the increases in testicular tumors were statistically significant at all dose levels. Statistical analysis by HED obtained essentially the same results, except for the low dose group which did not show pair-wise significance. However, statistical evaluations should not be considered to be the final word without any consideration of the biological relevance of the data. Historically for this tumor type, the spontaneous occurrence often approaches 100% by the end of a study.

Therefore, the Committee concluded that, in spite of the above statistical evidence, the testicular tumors are NOT treatment related since: (1) this non-lethal tumor was observed in nearly 100% of male rats including controls; (2) the apparent statistical significance of the tumor incidence at 6000 and 12,000 ppm [***Note***: both doses were determined to be excessive in males] could be attributed to the high mortality at these doses—resulting in earlier observation of the tumor—and significance was considered to be an artifact of the Peto's Prevalence Analyses protocol; (3) sufficient data are not available to determine if there was a decrease in the latency period [i.e., There was no serial sacrifice to determine latency. In fact, the first tumor occurred in the control group during week 54.]; and (4) this tumor type is not useful in overall evaluation since its occurrence is similar at all dose

levels.[11]

(ix). Mononuclear Cell Leukemia (Rat)

At the 15-October-1997 meeting the Committee evaluated the mononuclear cell leukemia and concluded that the occurrence of this tumor type in female rats is not attributable to treatment due to lack of statistical significance at any dose level and the incidences were within in the historical control range of the testing laboratory (15 to 36%). At the 24-February-1999 meeting, the Committee determined that additional statistical analysis using Peto's prevalence test was needed for this tumor type in male rats. Results of this analysis presented below in **Table 16a** were evaluated at the 23-June-1999 Committee meeting. At the 12-April-2000 meeting, the CARC reevaluated this lesion based on comments by Dr. Brian Dementi concerning historical control data and recommendations in the 30-March-2000 memorandum by Marion Copley titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion.**"**

Table 16a. Mononuclear Cell Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Male	23/55	16/55	24/55	17/53	1[a]/52
(%)	42	29	44	32	2
p=	-	-	0.463	-	-
Female	9/55	**18/55**	15/55	13/54	10[b]/55
(%)	16	**33**	27	24	18
p=	0.917	**0.025**[*]	0.059	0.181	0.670

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, July 16, 1997 & May 03, 1999).
[a] First mononuclear cell leukemia observed in a males at week 64, dose 12,000 ppm.
[b] First mononuclear cell leukemia observed in a female at week 47, dose 12,000 ppm
Note: Interim sacrifice animals are not included in this analysis. There were no mononuclear cell leukemia in any of the interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then p <0.05; If **, then p <0.01

[11] See item # 5 in Attachment 25, memorandum titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion" dated March 30, 2000 for a minority opinion expressed by Dr. Brian Dementi and the CARC response. Additional details can be found in Attachments # 1 - 22, written by Dr. Dementi.

Table 16b. Male rats (Fischer 344) with MCL that died from MCL

DOSE	control	100/50 ppm	500 ppm	6000 ppm	12,000 ppm
MCL as cause of death/# with MCL	7/23	7/16	14/24	13/18	1/1
% rats with MCL, dying from MCL	30	44	58	72	100

Table taken from the DER (MRID 43942901)
MCL - mononuclear cell leukemia

The Committee evaluated the evidence in **Table 16b** which suggested that there is an increase in leukemic animals dying from MCL with increasing dose. It was suggested that this may indicate an increase in severity of MCL (which would indicate increased carcinogenic response). Also presented at the meeting was the onset of this tumor in the various groups:

1st MCL observed in 12,000 ppm group - week 62
2nd MCL observed in 500 ppm group - week 72
3rd MCL observed in 6000 ppm group - week 74
4th MCL observed in 500 ppm group - week 82
5th, 6th MCL observed in 500 and 6000 ppm group - week 83
7th MCL observed in control group - week 84

Other data presented included the mean occurrence of death for those leukemic animals that died on study (includes only animals who died due to MCL; other animals were either sacrificed at term or death was attributed to other causes by the study pathologist):

control group 94.5 ± 7.2 weeks (22 animals), 1st death week 84
100/50 ppm group 94.3 ± 8.0 weeks (16 animals) 1st death week 82
500 ppm group 94.0 ± 10.0 weeks (24 animals) 1st death week 72
6000 ppm group 92.8 ± 8.3 weeks (18 animals) 1st death week 74
12,000 ppm group 62 weeks (only 1 animal) 1st death week 62

As can be seen in the previous two lists, there is little decrease in the onset of the first tumor, and the mean week of death is similar for all groups except the high dose where there was only one animal with MCL. Therefore, while more leukemic malathion treated animals appeared to die on study than leukemic controls, they do not appear to die earlier than leukemic controls dying on study (e.g., 94.5 ± 7.2 weeks for controls *vs.* 92.8 ± 8.3 weeks for 6000 ppm). The CARC concluded that the apparent increase in the number of leukemic animals dying from MCL was not indicative of increased severity or early onset.

The CARC also noted the absence of a dose-response relationship, and the incidences were within the historical control range of the testing laboratory (15 to 36%). Additionally, mononuclear cell tumors were not seen in three strains of rats: the Osborne-Mendel (1978 NCI-malathion); Sprague-Dawley (1980-FDRL-malathion); and F344 (1979, NCI-malathion and malaoxon and the 1996 malaoxon studies). However, the results of the old studies should

be used with caution to support or refute any results since the Cancer Peer Review Committee felt that there were many issues regarding the adequacy of each study**.**

The Committee concluded that there is no evidence for increased carcinogenicity based on MCL because: 1) this tumor occurs commonly in Fischer 344 rats and the incidences were within historical control ranges; 2) there was no statistical significance at any dose; 3) there was no dose response; 4) there was no indication of early onset or increased incidence. It was noted that attributing the cause of death to MCL is subjective and not a reliable indicator of increased severity.[12]

C. Non-Neoplastic Lesions

The nasal/oral tissue sections taken from five nasal regions from all rats were histopathologically examined. Treatment-related non-neoplastic lesions of the nasal mucosa were seen in both sexes at all dose levels including the controls (**Table 17**). In both sexes, lesions of the olfactory/respiratory mucosa were more severe at 500, 6000 and 12,000 ppm. Most of the non-neoplastic lesions did not occur in section 5, the section where the nasal tumors in females occurred.

At excessive doses there also was parathyroid hyperplasia. There were other histopathologic findings in the thyroid, lymph nodes, lungs, liver, spleen, adrenal gland and eyes at the top three doses in males and the top two doses in females.

[12] See item #47 in Attachment 25, memorandum titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion" dated March 30, 2000 for a minority opinion expressed by Dr. Brian Dementi and the CARC response. Additional details can be found in Attachments # 1 - 22, written by Dr. Dementi. However this issue was revisited by the CARC at the 12-April-2000 meeting.

Table 17. Non-neoplastic Lesions of the Nasal Mucosa in Male and Female Rats [a].

Type of Lesion	# of Sections Examined/Rat	0 ppm	100/50 ppm	500 ppm	6000 ppm	12,000 ppm
MALES						
Olfactory/Respiratory Mucosa: subacute (chronic active)/ chronic inflammation	3	3/8	2/14	32/23	113/63	55/27
Olfactory Epithelium, Degeneration/Atrophy	3	4	3	14	197	150
FEMALES						
Olfactory Mucosa Congestion Respiratory Mucosa Congestion	5	10 25 (3)	11 27 (0)	16 46 (4)	25 40 (0)	50 70 (2)
Olfactory Edema Respiratory Edema	4	0 0	1 4	3 9	67 28	45 38
Squamous/Squamoid Metaplasia, Focal Multi-Focal	 3 2	 0 1	 4 4	 0 7	 3 8	 0 0
Olfactory/Respiratory Mucosa: subacute (chronic active) /chronic inflammation), Multi-Focal	4	0/5	0/28	2/7	2/11	4/6
Nasal Mucosa: Olfactory Epithelium, Degeneration/Atrophy	3	0	6	5	239	150
Nasal Mucosa: Olfactory Epithelium, Degeneration / Atrophy, Multi-Focal	3	2	1	1	26	103
Paranasal Sinus(es): Maxillary Gland-Atrophy	2	1	0	19	45	13
Nasal Mucosa (Vestibular), Congestion	1 (1)	8 (8)	12 (12)	18 (18)	16 (16)	33 (33)
Nasal Mucosa (Vestibular), Squamous Cell Hyperplasia	1 (1)	1 (1)	0	1 (1)	7 (7)	2 (2)
Nasal Mucosa (Vestibular), Squamous Cell Hyperplasia, Focal	1 (1)	1 (1)	2 (2)	1 (1)	1 (1)	1 (1)
Nasal Mucosa (Vestibular), Squamous Cell Hyperplasia, Multi-Focal	1 (1)	1 (1)	8 (8)	5 (5)	10 (10)	0

a =Incidences are the total of the lesions seen in all sections of the nasal mucosa. Essentially 90 animals/group were examined for all tissues except for section 5 where 78-81/group (males) and 78-85/group (females) were examined. ()=the number of lesions in section 5. Most of the inflammatory lesions did not occur in section 5, the section where the nasal tumors in females occurred.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Mortality was statistically significantly increased in males at 6000 ppm and in both sexes at 12,000 ppm. Statistical analyses of mortality data are presented in **Tables 18 and 19** for males and females, respectively. Mortality was not attributed to liver tumors. In males at 500 ppm there was a non-statistical, but probably biologically significant increase when compared to controls (47% as compared to 33% in controls). There was a significant increasing trend with increasing doses of malathion in males and females. Males at 12,000 ppm had 100% mortality at week 97. Although there was increased mortality in males at 6000 ppm, there were enough rats "at risk" to evaluate carcinogenic potential. Decrements in body weight gain were 13% in males and 4% in females at 6000 ppm and 32% in males and 15% in females at 12,000 ppm. Both sexes of rats at 6000 and 12,000 ppm exhibited anemia. The statistically significant ($p < 0.01$) cholinesterase inhibition in plasma, red blood cell and brain observed at 6000 and 12,000 ppm are summarized in **Table 20.**

Table 18. Male Rat Mortality Rates[+] and Cox or Generalized K/W Test Results.

Dose (ppm)	Study Weeks						
	1-26	27-53	54 [i]	54-78	79-106[f]	Total	Percentage
0	0/70	0/70	15/70	0/55	18/55	18/55	**(33)****
100/50	0/70	0/70	15/70	0/55	14/55	14/55	(25)
500	0/70	0/70	15/70	3/55	23/52	26/55	(47)
6000	0/70	0/70	15/70	1/55	38/52 [a]	39/53	**(74)****
12,000	1/70	1/69	14/68	15/54	39/39	56/56	**(100)****

[+] Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[i] Interim sacrifices at week 14, 27 and 54. Only week 54 interim sacrifice included in this analysis.
[f] Final sacrifice at week 105
[a] Two accidental deaths at weeks 105, dose 6000 ppm
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 19. Female Rat Mortality Rates[+] and Cox or Generalized K/W Test Results.

Dose (ppm)	Study Weeks						
	1-26	27-53	54[i]	54-78	79-106[f]	Total	Percentage
0	0/70	0/70	15/70	1/55	16/54	17/55	**(31)****
100/50	0/70	1/70	14/69	1/55	13/54	15/56	(27)
500	0/70	0/70	15/70	2/55	12/53	14/55	(25)
6000	0/70	1/70	15/69	1/54	19/53	21/55	(38)
12,000	0/70	1/70	15/70	4/55	30/51	35/55	**(64)****

[+] Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[i] Interim sacrifices at week 14, 27 and 54. Only week 54 interim sacrifice included in this analysis.
[f] Final sacrifice at week 105
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 20 Cholinesterase Activity in Rats Fed Malathion for 24 Months.

Percent Cholinesterase Inhibition in Rats At 24 months						
Dose	500 ppm		6000 ppm		12000 ppm	
Sex	Male	Female	Male	Female	Male	Female
Plasma	29**	18	64**	61**	Dead	89**
RBC	17	27**	43**	44**	Dead	52**
Brain	3	1	21**	18**	Dead	67**

Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then $p < 0.05$; If **, then $p < 0.01$

The Committee further evaluated the acute and subchronic studies. Data from these studies showed that the cholinesterase inhibition seen at 6000 ppm in the chronic study was not supported by the cholinesterase inhibition observed at a comparable dose (5000 ppm) in the 90-day study or at 2000 ppm in the acute study. The Committee concluded that the 12,000 ppm dose in both sexes (due to severe cholinesterase inhibition) and the 6000 ppm

dose in males (due to mortality) were excessive. It was determined that the 500 ppm dose in males was adequate to assess the carcinogenic potential of malathion based on a non-statistically, but biologically significant increase in mortality at this concentration (47% as compared to 33% in controls); and a decrease in plasma cholinesterase (29%, $p \leq 0.01$). In females,, the 6000 ppm dose was considered adequate based on a decrease in plasma, RBC and brain cholinesterase (61, 44 and 18 %, respectively). This dose was one-half the next dose where mortality was increased.[13]

3. Combined Chronic Toxicity/Carcinogenicity Study with Malaoxon in Fischer 344 Rats

Reference: Daly, W. I.: "A 24-Month Oral Toxicity/Oncogenicity Study of Malaoxon in the Rat via Dietary Administration", April 2, 1996. , Lab. Study No.: 93-2234, Testing Facility: Huntingdon Life Sciences, East Milestone, NJ (MRID 43975201).

A. Experimental Design

Malaoxon technical (96.4% a.i.) was administered in the diet to groups of 85 male and female F344 rats at 0, 20, 1000 and 2000 ppm [equivalent to 0, 1, 57, and 114 mg/kg/day (males) and 0, 1, 68 and 141 mg/kg/day (females)] for 2 years. Ten rats/sex/group were sacrificed at 3 months, 6 months, and 12 months for interim evaluations and cholinesterase activity determinations. There were 55 rats/sex/group devoted to the full 2-year study.

B. Discussion of Tumor Data

As shown in **Table 21,** there was a statistically significant ($p < 0.05$) increase in mononuclear cell leukemia in male rats at the highest dose tested (2000 ppm). There was also a statistically significant trend ($p < 0.05$) for these tumors.

Table 21. Mononuclear Cell Leukemia in Rats Fed Malaoxon for 24 Months.

Sex	0 ppm	20 ppm	1000 ppm	2000 ppm
Males	13/55 (24%) **p=0.03***	12/55 (22%)	19/55 (35%) p =0.07	16/55 (29%) **p =0.05***
Females	8/55 (15%)	9/55 (16%)	10/55 (18%)	5/55 (9%)

Method of Peto, *et al*. (1980) per September 22, 1997 letter of Huntington Life Sciences to Dr. Judy Hauswirth.

[13] See item # 9 in Attachment 25, memorandum titled, "Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion" dated March 30, 2000 for a minority opinion expressed by Dr. Brian Dementi and the CARC response. Additional details can be found in Attachments # 1 - 22, written by Dr. Dementi. However this issue was revisited by the CARC at the 12-April-2000 meeting.

The Committee concluded that the mononuclear cell leukemia is NOT treatment related since statistical significance was seen only in males at a dose that was determined to be excessive, there was no dose-response, and the incidences were within the historical control range (15 to 36%) of the testing laboratory. Additionally, mononuclear cell tumors were not seen in three strains of rats: the Osborne-Mendel (1978, NCI-malathion); Sprague-Dawley (1980, FDRL-malathion), Fischer 344 (1979, NCI-malathion and malaoxon). However, the results of the old studies should be used with caution to support or refute any results due to inherent problems in these studies.

C. Non-Neoplastic Lesions

Nasal lumen inflammation was seen in high dose males and in mid and high dose females. Nasal lumen epithelial hyperplasia was increased in mid and high dose females. Lung interstitium inflammation was increased in mid and high dose females and tympanic cavity inflammation was seen in mid and high dose early female decedents. Increased incidences of mineral deposits in the stomach muscularis were seen in mid and high dose males.

D. Adequacy of Dosing for Assessment of Carcinogenicity

Mortality was significantly ($p<0.01$) increased at the high dose (2000 ppm) in males (53%) and females (49%) compared to controls (males, 29% and females, 13%). There was severe inhibition of cholinesterase activity for all three compartments (plasma, RBC and brain) in both sexes at 1000 and 2000 ppm at various time points during treatment compared to controls. At 1000 ppm, cholinesterase inhibition was: plasma, 74 to 81% in males and 82 to 87% in females; RBC, 54 to 66% in males and 45 to 62% in females; and brain, 2 to 30% in males and 5 to 14% in females. At 2000 ppm cholinesterase inhibition was: plasma 83 to 91% in males and 90 to 96% in females; RBC, 56 to 65% in males and 54 to 66% in females; and brain, 11 to 74% in males and 61 to 78% in females. The Committee concluded that 2000 ppm was excessive based on increased mortality and severe cholinesterase inhibition in all three compartments, and 1000 ppm adequate to assess carcinogenic potential because it has some evidence of ChE inhibition and was one-half the dose causing excessive toxicity.

IV. MUTAGENICITY

Three acceptable studies [*S.typhimurium/E. coli* reverse gene mutation assay, *in vivo* bone marrow cytogenetic assay in rats, and an unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes] were available for review. Findings from the submitted guideline studies indicate that malathion did not cause gene mutations in bacteria or UDS in cultured rat hepatocytes. Similarly, malathion was neither clastogenic nor aneugenic up to doses that showed clear cytotoxicity for the target tissue *in vivo*. Studies from the open literature indicated that malathion was positive both in vitro and *in vivo*. However, there are uncertainties regarding the relevance of these findings to a mutagenic mode of action for malathion since positive results both *in vivo* and *in vitro* were seen at cytotoxic doses and the types of induced aberrations were asymmetric (i.e., chromatid and chromosome breaks and

exchanges) and, therefore, not stable. Nevertheless, malathion was shown to be weakly reactive with DNA and contain a structure that suggests electrophilicity. **The Committee concluded, however, that the weight of the evidence supports neither a mutagenic hazard nor a role for mutagenicity in the carcinogenicity associated with malathion.** Summarized findings supporting the above conclusions are presented below:

A. Gene Mutation

In a *Salmonella typhimurium/Escherichia coli* reverse gene mutation assay, malathion (94.5%) was non-mutagenic when tested at concentrations up to 5000 µg/plate (highest dose) with or without metabolic (S9) activation (MRID No. 40939302).

B. Chromosome Aberrations

In an *in vivo* bone marrow cytogenetic assay, malathion (94%) was negative following oral doses at 500-2000 mg/kg to male and female Sprague-Dawley rats. A dose-related reduction in mitotic indices (MIs) was seen in the females of all treatment levels at 24 hours. Reduced MIs were also recorded for high-dose males and females at 48 hours (MRID No. 41451201).

C. Other Mutagenic Effects

In an *in vitro* primary rat hepatocytes unscheduled DNA synthesis (UDS) assay, malathion (94%) was negative up to cytotoxic levels ($\geq$ 0.12 µL/mL; $\approx$ 150 µg/mL) (MRID No. 41389301).

D. Other Information

An open literature review of the mutagenicity studies on malathion and malaoxon, the major metabolite formed by oxidation, was prepared for the Carcinogenicity Peer Review of Malathion held on 7-February-1990.

The overall assessment, indicating positive clastogenicity, should be interpreted with caution. While five of the seven *in vivo* bone marrow studies were reported positive by Flessel *et al.* (1993), evidence of structural chromosome damage was either accompanied by cytotoxic effects (i.e., significantly reduced mitotic indices or increased cell cycle delay) or asymmetrical structural aberrations (i.e., chromatid and chromosome breaks and exchanges). Questions also arose regarding the purity of the test material. A similar observation regarding cytotoxicity and the induction of unstable aberrations, which generally lead to death and hence do not directly contribute to carcinogenesis, can also be made for the 5/6 positive *in vitro* cytogenetic assays. Weak but positive results were shown for sister chromatid exchange induction at high, cytotoxic doses (Galloway *et al.*, 1987) and for methylation in a submitted metabolism study (MRID No. 41367701). No assays with germinal cells have been submitted to the Agency. However, malathion was negative in *Drosophila melanogaster* sex linked recessive lethal assay, mouse dominant lethal assays and spermatogonia/or spermatocyte

cytogenetic assays. A questionable clastogenic response was reported in mouse spermatocytes following subacute exposure to commercial grade malathion (Salvadori *et al.*, 1988). Nevertheless, the data from developmental and reproduction studies, as well as epidemiological surveys of pregnant women exposed to malathion (Arevalo *et al.*, 1987; Spielman, 1986; Grether *et al.*, 1987), do not suggest an adverse heritable effect.

No mutagenicity studies have been submitted to OPP on malaoxon. The consensus opinion from the above cited reviews of the open literature is that malaoxon is not mutagenic in bacteria but is a confirmed positive without S9 activation in the mouse lymphoma forward gene mutation assay. Malaoxon was not clastogenic in cultured Chinese hamster ovary (CHO) cells; however, the findings from the mouse lymphoma assay suggest that malaoxon may induce both gene mutations and chromosome aberrations. Malaoxon has a structure similar to malathion and, therefore, concerns for possible electrophilicity may also apply, despite the evidence of no carcinogenicity.

E. Conclusions:

Results of the guideline genetic toxicology studies with malathion indicated that the test material did not cause gene mutations in bacteria or UDS in cultured rat hepatocytes. Similarly, malathion was neither clastogenic nor aneugenic up to doses that showed clear cytotoxicity for the target tissue *in vivo*. The CARC included that *in vitro* and *in vivo* findings from the open literature should be interpreted with caution since positive results were seen at cytotoxic doses and/or the types of induced aberrations were asymmetric and, therefore, not consistent with cell survival. The question of test material was also an issue. Although the structure of malathion suggests electrophilicity, the Committee concluded that the weight of the evidence supports neither a mutagenic hazard nor a role for mutagenicity in the carcinogenicity associated with malathion.

V. STRUCTURE-ACTIVITY RELATIONSHIP

Both malathion and malaoxon should be considered structural analogs of each other.

VI. TOXICOLOGY

A. Acute Toxicity

In acute toxicity studies conducted in rats, malathion exhibits low acute toxicity potential via the oral (LD50 = 5500 mg/kg), dermal (LD50 >2,000 mg/kg) and the inhalation (LC50 of >5.2 mg/L) routes.

In an acute neurotoxicity study, groups of Sprague-Dawley rats (27/sex/dose) received a single oral administration of malathion (96.4%) in corn oil at doses of 0, 500, 1000, or 2000 mg/kg. For neurotoxicity, the NOAEL was 1000 mg/kg and the LOAEL was

2000 mg/kg/day based on decreased motor activity at peak effect time (day 1) and clinical signs (salivation, body staining, tremors in one animal, labored breathing, stained fur, decreased defecation and urination). Plasma and RBC cholinesterase were inhibited in both sexes at 2000 mg/kg. Also, there was an equivocal inhibition of plasma cholinesterase for females at 500 and 1000 mg/kg, characterized by a poor dose response. No inhibition of brain cholinesterase activity was seen in either sex at any dose level. Equivocal neuropathological findings at 2000 mg/kg included axonal degeneration in the lumbar root and bilateral retinal rosette in one male, digestion chambers in the lumbar dorsal root fibers in one male and in the sciatic and tibial nerve in another male rat. Digestion chambers and axonal degeneration of the sciatic nerve were also seen in one male control rat (MRID No. 43146701).

B. Subchronic Toxicity

In a subchronic neurotoxicity study, groups of Sprague-Dawley rats (25/sex/dose) were fed diets containing malathion (96.4%) at 0, 50, 5000 or 20,000 ppm (0, 4, 352, or 1486 mg/kg/day in males and 0, 4, 395, or 1575 mg/kg/day in females, respectively). For systemic toxicity, the NOAEL was 5000 ppm (352/395 mg/kg/day for M/F) and the LOAEL was 20,000 ppm (1486/1575 mg/kg/day in M/F) based on decreased body weight and food consumption and on increased clinical signs (anogenital staining, and dried red material around the nose). For cholinesterase inhibition, the overall NOAEL was 50 ppm (4 mg/kg/day) and the LOAEL was 5000 ppm (352/395 mg/kg/day in M/F) based on inhibition of plasma and red blood cell cholinesterase in males and females and on brain cholinesterase in females. There were no treatment-related effects on brain weight or neuropathology (MRID No. 43269501).

In a subchronic inhalation study, groups of Sprague-Dawley rats (15/sex/concentration) were exposed in whole body inhalation chambers to malathion (96.4%) at aerosol (analytical) concentrations of 0.1, 0.45, or 2.01 mg/L for 6 hours/day, 5 days/week for 13 weeks. Treatment had no effects on survival, body weights or food consumption. Cholinergic signs observed at 2.01 mg/L and sporadically in a few animals at the lower doses included red staining of the urogenital areas, excess salivation and ungroomed oily fur. Treatment-related histopathological lesions were seen in the nasal cavity and the larynx of both sexes of rats at all concentrations tested. The lesions in the nasal cavity were characterized as slight to moderate degeneration and/or hyperplasia of the olfactory epithelium which was locally extensive. The lesions of the larynx were characterized as epithelial hyperplasia, with squamous keratinization occurring in some rats. In addition, the olfactory/respiratory epithelial junction was severely affected in most animals. For systemic toxicity, a NOAEL was not established and the LOAEL was 0.1 mg/kg/day based on histopathologic lesions of the nasal cavity and larynx. Inhibition of plasma and red blood cell cholinesterase activity was seen in females rats at all concentrations. In male rats, inhibition of cholinesterase activity was observed in plasma at 2.01 mg/L and in red blood cells at $\geq$ 0.45 mg/L. Inhibition of brain cholinesterase activity was seen only at the highest concentration. A NOAEL was not established for plasma and RBC cholinesterase inhibition; the LOAEL was 0.1 mg/L. For inhibition of brain cholinesterase, the NOAEL was 0.45 mg/L and the LOAEL was 2.01 mg/L (MRID No. 43266601). The HIARC has requested for another

subchronic inhalation toxicity study due to the lack of a NOAEL for cholinesterase inhibition as well as non-neoplastic lesions in this study (See HIARC Report dated 12/28/98).

C. Chronic Toxicity

In a combined chronic toxicity/carcinogenicity study in rats (discussed earlier), mortality was increased in males at 6000 ppm and in both sexes at 12,000 ppm. There was a significant increasing trend with increasing doses of malathion in males and females. Male rats at 12,000 ppm had 100% mortality at week 97. Decrements in body weight gain were 13% in males and 4% in females at 6000 ppm and 4% in males and 15% in females at 12,000 ppm. Both sexes of rats at 6000 and 12,000 ppm exhibited anemia. Significant inhibition of plasma, RBC and brain cholinesterase activity was seen in both sexes at 6000 and 12,000 ppm. Based on the re-assessment of the nasal tissues, for males, the NOAEL was 100/50 ppm and the LOAEL was 500 ppm based on non-neoplastic lesions of the nasal mucosa; a NOAEL was not identified for females (MRID No. 43942901; 44782301).

In a carcinogenicity study in B6C3F1mice (discussed earlier), mortality rates, clinical signs of toxicity and hematological parameters were not affected by treatment with malathion at any dose. There were decreased absolute body weights at 8000 and 16,000 ppm in both sexes, ranging 14.3-20.0% in males and 9.7-16.1% in females throughout the entire duration of the study. The NOAEL for plasma and RBC cholinesterase inhibition was 100 ppm, and that for brain cholinesterase inhibition was 800 ppm for both sexes (MRID No. 43407201).

D. Metabolism

In a metabolism study in Sprague-Dawley rats, single doses of radiolabeled 14C-malathion (98% purity; SA = 90.0 uCi/mg) were administered by oral gavage to groups of 5 male and 5 female adult rats at dose levels of 40 mg/kg (low dose), 800 mg/kg (high dose) and 40 mg/kg following 15 days of daily oral gavage of non-radiolabeled malathion (94.6% purity) at a dose level of 40 mg/kg/day. The rats were then placed in metabolism cages and urine and feces were collected for 72 hours. Radioactivity in urine and feces was determined at 4, 8, 12, 24, 48 and 72 hours after dosing. In a preliminary study, it was determined that less than 1% of the radioactivity in similarly treated animals was eliminated in expired air. At 72 hours, the animals were sacrificed and major organs/tissues (including GI tract plus contents and residual carcass) were collected, weighed and analyzed for radioactivity. Whole blood, plasma and RBCs were also analyzed for radioactivity. In addition, individual and pooled urine and fecal samples were analyzed for biotransformation products (i.e., malathion and metabolites) at 0-24 hours and 24-48 hours after dosing (MRID No. 41367701).

More than 90% of the radioactivity in the 40 mg/kg low dose was excreted within 72 hours with most excretion occurring in the first 24 hours and considerably less occurring during the remainder of the 72 hour period. Approximately 80-90% of the radioactivity in the administered dose was excreted in the urine with females excreting slightly more than males in the urine. Only minor differences in urine/fecal excretion ratios were observed between animals given 40 mg/kg (low dose), 800 mg/kg (high dose) and 40 mg/kg after 15 previous

daily doses of malathion. At 72 hours, the highest concentration of radioactivity was observed in the liver, but less than 0.3% of the administered radioactivity was present in that organ. Radioactivity did not bioaccumulate in any of the organs/tissues analyzed. Although 8 radiolabeled metabolites were observed in urine, greater than 80% of the radioactivity in urine was represented by the diacid (DCA) and monoacid (MCA) metabolites. The remaining radiolabeled metabolites were identified as components of "peak A" and "peak B". It was determined that between 4 and 6% of the administered dose was converted to malaoxon, the active cholinesterase inhibiting metabolite of malathion (MRID No. 41367701).

VII. RESPONSES TO CHEMINOVA

Cheminova submitted comments (in a letter dated 29-March-2000, from Jellinek, Schwartz & Connolly, Inc) on EPA's Risk Assessments for Malathion. The CARC evaluated only those comments (listed below) that relate directly to the evaluation of the carcinogenic potential of malathion.

Issues include:

A. New Pathology Working Group Review

The CARC accepted the results of the PWG reevaluation of the liver slides and is using the new tumor incidences in the weight of evidence. The CARC concluded that the PWG was conducted in accordance with FR Notice 94-5 and the new values for liver tumors presented in **Table 7b** should be used. **Table 7c** presents the comparison of diagnoses among the original study pathologist, peer review pathologist and the PWG consensus. There was discussion regarding the occurrence of cellular alteration. However, it was determined that: 1) cellular alteration is not a reliable indicator of progression to neoplasia, and 2) there was no basis for considering this to be a preneoplastic lesion in this study since there was no increase of basophilic foci (based on the original study report).

B. Cheminova's Concerns about CARC's Assessment of Malathion Chronic Bioassays

1. Female rat liver tumors - The CARC has already accepted the PWG report and agrees that there is an increased incidence of hepatocellular adenomas only at 12,000 ppm, a dose with excessive toxicity.

2. Rat nasal tumors - The CARC has reevaluated these tumors and concluded that it can not be determined whether they are due to treatment or random occurrence. It should be noted that in the females, the 2 tumors occur in section 5, a section where these is little evidence of inflamation in the nasal mucosa. The CARC does not feel that a possible systemic effect can be excluded. A discussion of the historical control data is in the body of this report.

3. Mouse oncogenicity study - There is no disagreement with Cheminova's statement that "…there is no evidence of carcinogenicity in the mouse at levels below those causing excessive toxicity."

4. Other studies should be taken into account - The CARC routinely considers all available data when evaluating the weight of the evidence. It should be noted that the CARC does not routinely "**discard or discount**" doses where there is evidence of excessive toxicity. This information is considered together with the remainder of the data base as required by the draft cancer guidelines. The weight that is placed on tumors that occur at these doses depends on what else is observed in the data base.

C. Cheminova's Concerns about CARC'S Genotoxicity Assessment

1. Comment: The representative (Jellinek, Schwartz & Connolly, Inc) on behalf of the registrant (Cheminova, A/S) agreed with the Cancer Assessment Review Committee (CARC) that results of guideline studies with malathion were negative.

Response: No comment

2. Comment: Jellinek, Schwartz & Connolly, Inc., disagreed with the Agency's use of the phrase: "overwhelming confirmation from the published literature demonstrating that malathion is genotoxic..."

Response: The publication in question (Flessel *et al*., 1993) was cited in Section IV., D. (Mutagenicity, Other Information) of the CARC's assessment of the genotoxicity of malathion. This overview of the genetic toxicology of malathion, along with other available literature was used to draw the conclusion that malathion was clastogenic both *in vitro* and *in vivo* in the earlier cancer peer review of malathion (September 24 and October 8 and 15, 1997). At the time this document was prepared, information regarding the role of cytotoxicity in false positive cytogenetic assays, which was presented at the March 1999 International Workshop on Genotoxicity Test Procedures and has been recently published (Galloway, 2000), was not available to the Committee. In light of this information, the issue of the clastogenicity of malathion was revisited at the June 23, 1999 CARC meeting. Based on a review of the articles in the Flessel *et al.* publication, it became clear that while technical malathion was clastogenic as stated in the document:

> " It should be noted, however, that while 5 of the 7 *in vivo* bone marrow studies were reported positive, evidence of structural chromosome damage was either accompanied by cytotoxic effects (i.e., significantly reduced mitotic indices or increased cell cycle delay) or asymmetrical structural aberrations (i.e., chromatid and chromosome breaks and exchanges). A similar observation regarding cytotoxicity and the induction of unstable aberrations, which generally lead to death and hence do not directly contribute to carcinogenesis, can also be made for the 5/6 positive *in vitro* cytogenetic assays."

It appears, however, that the attempt by the Committee to reduce the level of concern for the clastogenicity of malathion was not clearly presented. To eliminate confusion, the phrase: "overwhelming confirmation from the published literature demonstrating that malathion is genotoxic..." will be replaced in the revised document with:

"The overall assessment indicating positive clastogenicity should be viewed with caution."

3. Comment: Jellinek, Schwartz & Connolly, Inc. claimed that the CARC relied solely on the Flessel *et al*. review article and not on the primary references in reaching the conclusion about the mutagenicity/clastogenicity of malathion.

Response: On the contrary, the conclusion that positive results were obtained at cytotoxic doses and the induction of unstable structural chromosome cast doubts on the relevance of the findings comes from a review of the individual studies.

4. Comment: Jellinek, Schwartz & Connolly, Inc. believe that much greater weight should be given to the guideline studies.

Response: High confidence is given to the acceptable guideline studies. However, HED considers all of the available data (submitted and published) in a weight-of-the-evidence (WOE) approach. In the interest of public health, the CARC will continue to use both the guideline studies and the data from the open literature to ensure that a complete and through analysis of the test material is prepared. This approach will provide the risk assessors the opportunity to make informed decisions in the risk assessment.

5. Comment: Jellinek, Schwartz & Connolly, Inc. believe that the electrophilicity issue raised by the CARC for malathion is irrelevant.

Response: We disagree with this comment. The role of the CARC is to look at all of the available data and particularly, note areas of concerns and/or uncertainties and list reasons for this concern. The malathion issue of electrophilicity is a good example of the application of the WOE approach used by the CARC.

The methyl group of malathion and other OPs is slightly polarized and can behave as an electrophile (Preussman *et al*., 1969; Bedford and Robinson, 1972). In addition to cleavage by hydrolysis, this methyl group is known to undergo nucleophilic cleavage by GSH (with production of methylated GSH) as in the case of dichlorovos and methyl parathion (Chambers *et al*., 1995). Our revisit of the malathion rat metabolism study (MRID No. 41367701, dated December 20, 1989) indicates that malathion undergos demethylation by GSH. Furthermore, it has been noted in the literature (Chambers *et al*., 1995; Ryan and Fukuto, 1984, 1985) that malathion undergoes O-demethylation in rats via nucleophilic attack by GSH. From this information, one may conjecture that other cellular nucleophiles, (e.g. bases on DNA), may also be methylated by malathion. The CARC believes that there is nothing in the above rationale that contradicts Woo *et al*., (1996) comments on the structure-activity relationships of alkyl phosphates/phosphonates:

> "Until proven otherwise, virtually all fully esterified alkyl (methyl, ethyl, and, to a lesser extent, propyl) phosphates/phosphonates and their thio derivatives should be suspect to be of some carcinogenic concern because of their expected alkylating activity."

The inclusion of the Ashby and Tennant (1991) article, which post-dates the reference

cited by the registrants's representative, was intended only as additional support regarding electrophilicity. However, even if this reference is removed, we continue to have concerns. To put these concerns into a proper perspective, relative to the available mutagenicity data for malathion, the following statement will be added to the revised document.

> " The Committee concluded that the weight-of-the-evidence neither supports a mutagenic hazard nor a role for mutagenicity in the carcinogenicity associated with malathion."

6. Comment: Jellinek, Schwartz & Connolly, Inc. claimed that the methyl groups on malathion are "quite stable, unreactive moieties and are highly unlikely to be alkylating agents."

Response: The CARC would like to see the data supporting this assertion and refers the commentor to our response to Comment 5.

D. Epidemiology

CARC does not feel that the information provided would alter the cancer classification of "suggestive."

A study of "Mortality and Incidence of Cancer Among Employees at Cheminova Agro" was conducted by the Danish Institute for Clinical Epidemiology and the Danish Cancer Society at the request of Cheminova's Works and Safety Council. Cheminova Agro is a company that produces insecticides, herbicides, mining chemicals, and preservatives. The study examined medical records and death certificates for all staff employed for at least one year from 1953 to 1993. The total cohort consisted of 1,467 persons including 1,275 men and 192 women. Based on an earlier survey, there were 583 men with high exposure to organophosphate insecticides, from 1953 to 1983. The low exposure group consisted of laboratory technicians, engineers, warehouse staff, canteen staff, and clerical staff. Subsequent to 1983, 359 workers were classified as blue collar and 220 as white collar. Presumably white collar workers had less exposure to organophosphates. The exact number with high exposure to malathion is not reported. There were 160 deaths involving 158 men and 2 women. Mortality and cancer incidence were compared with the Danish population taken as a whole with adjustments for differences in sex and age.

The Standardized Mortality Ratio (SMR) among Cheminova Agro male employees was 4% less than the mortality for Denmark as a whole (SMR=96). Male employees with high exposure, employed prior to 1983, had a non-significant SMR of 1.04 (95% confidence interval 0.86-1.25). Analysis by specific cause of death (e.g., lung cancer, respiratory disease) did not reveal any significant increases for the cohort. However, only 70 cases died of cancer and, therefore, the confidence intervals were often relatively wide, permitting analysis of only the more common types of cancer (e.g., lung, colorectal). Examination of medical records revealed 87 cases of cancer incidence in the cohort. Of the 21 cancer sites analyzed, only one, saliva gland, had a statistically significant excess (based on 2 observed cases). However, given the number of analyses this could be a chance finding. The study authors concluded "with such small numbers it is difficult to form conclusions." Data were also analyzed by length of

employment, period of employment, and age group. However, none of these additional analyses revealed any additional significant findings. Overall, cancer incidence was about what would be expected based on a comparison with the general population of Denmark.

In conclusion the Danish study did not reveal any increase in mortality or cancer incidence that could be attributed to their exposures. It appears that only about half of the employees may have had significant exposure to organophosphate insecticides and no measurements were provided to assess the level of those exposures. Also, there was no measurements of the exposures to specific organophosphates (e.g., parathion, malathion). Given the limited period of follow up, the relatively small numbers employees with significant exposure, and the lack of measured exposure to malathion, this study should not be used to draw conclusions about the presence or absence of risk of cancer from exposure to malathion.

E. Weight of Evidence

Based on the PWG liver reevaluation and a reevaluation of the oral, nasal and mutagenicity data, the CARC has revised the weight of evidence and cancer classification from "likely" to "suggestive."

VIII. COMMITTEE ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

The Committee's assessment of the weight-of-the-evidence is presented below:

1. Carcinogenicity

Evidence for carcinogenicity was demonstrated by the presence of liver tumors in male and female B6C3F1 mice and female Fischer 344 rats at toxic doses. It could not be determined whether nasal and oral tumors in female Fischer 344 rats and one nasal tumor in a male rat were treatment-related or due to random occurrence.

A. Liver Tumors

In **male mice** (based on the PWG re-read), there was a positive trend (p=0.000) for **liver** adenomas and the combined tumors (adenomas/carcinomas). The incidence of adenomas was significantly increased at 8000 ppm (14/55, 25%, p = 0.0103) and 16,000 ppm (49/51, 96%, p = 0.000) when compared to controls (4/54, 7%). Similarly, the combined tumors (adenomas/carcinomas) showed pair-wise significance at 8000 ppm (15/55, 27%, p=0.006) and 16,000 ppm (49/51, 96%, p=0.000) when compared to controls (4/54, 7%). Although carcinomas were seen at 100 ppm, 800 ppm and 8000 ppm compared to zero in the controls, none of the incidences exhibited statistical significance nor was there a dose-related increase at any level.

When compared with the historical control ranges, the incidences of adenomas at 8000 ppm (25%) and 16,000 ppm (96%) exceeded the historical control range (14 to 22%)

(data for mean incidences are not available). The incidences of carcinomas at 800 ppm (5%) and 8000 ppm (4%) were within the historical control range (0 to 6.4%). No carcinomas were seen at 16,000 ppm while the incidence of carcinomas at 100 ppm (7%) was slightly outside the historical control range.

In **female mice**, there was a positive trend (p=0.000) for **liver** adenomas and the combined tumors (adenomas/carcinomas). The incidence of adenomas was significantly increased at 8000 ppm (9/52, 17%, p = 0.001) and 16,000 ppm (42/51, 82%, p = 0.000) when compared to controls (0/55). Similarly, the combined tumors (adenomas/carcinomas) showed pair-wise significance at 8000 ppm (10/52, 19%, p=0.003) and 16,000 ppm (43/51, 84%, p=0.000) when compared to controls (1/55, 2%). No statistically significant increases in carcinomas alone were seen at any dose level.

The Committee concluded that there is evidence of carcinogenicity in both sexes of mice at the two highest doses tested although these doses were considered excessive. There is no evidence of carcinogenicity in male or female mice at the lower doses.

In **female rats** (based on the PWG re-read), an increased incidence of **liver** adenomas was seen only at the highest dose tested, 12,000 ppm. This dose was considered to be excessively toxic based on severe inhibition of plasma (89%), RBC (52%) and brain (67%) cholinesterase activity and increased mortality (64%).

Statistical significance included a positive trend (p=0.005) for adenomas and pair-wise comparison for adenomas at 12,000 ppm (5/38, 13%, p = 0.009) when compared to controls (0/41). There were no carcinomas observed at any dose level.

When compared to the historical control data of the testing laboratory, the incidence of adenomas at 12,000 ppm (13%) dose exceeded the historical control range (0 to 5%) and mean (1.6%). In addition, the incidence of this tumor type exceeded the historical control incidence of the National Toxicology Program, report 1998 (0/901, 0.44%).

The Committee concluded that although the incidence of liver tumors in female rats was observed only at an excessively toxic dose (12,000 ppm), it provided evidence of carcinogenicity because: 1) the incidence was statistically significant by pair-wise comparison; 2) there was a statistical trend; 3) the incidence was outside the range of both the testing facility and NTP historical control data bases. It was also observed that: 1) this increase only occurred at an excessively toxic dose; and 2) there was no evidence of liver carcinogenicity in male rats at any dose level, but the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12,000 ppm, where mortality was 74% and 100%, respectively. There is, however, no evidence to either support or refute this supposition.

B. <u>Nasal Tumors</u>

In **male rats**, there was an **adenoma of the olfactory epithelium** at 6000 ppm and

an **adenoma of the respiratory epithelium** at 12,000 ppm compared to none in the controls. In **female rats,** there was an **adenoma of the respiratory epithelium** at 6000 and at 12,000 ppm compared to zero in the controls.

When compared to the historical control data base of the testing laboratory, the incidence of adenomas in this study (1 at 6000 ppm and 1 at 12,000 ppm) in both sexes exceeded the historical control incidence (0/240 males and 0/240 females). In addition, the NTP reported respiratory tract tumors in the respiratory epithelium of 6 of 4000 male rats and in the olfactory epithelium of 0 of 4000 males. Of the 6/4000 in the respiratory epithelium, four of the six were squamous cell tumors. Therefore, the relevant historical control incidence for the tumor type (adenomas) in question is 2/4000 control males.

Of the four nasal tumors, one in each sex at the two highest doses (6000 and 12,000 ppm), only the tumor at 6000 ppm in the females was at a dose that was not considered excessive. The Committee postulated that direct contact with malathion (by volatilization from the feed or by inhalation of the feed through the nose) was a possible explanation for the nasal tumors. However, there was no evidence to support or refute that the tumorigenicity was due to exposure by the inhalation or systemic route. Therefore, the Committee concluded that a systemic effect could not be unequivocally ruled out.

The Committee concluded that it could not determine whether nasal tumors were either treatment-related or due to random occurrence. On the one hand: (1) there was no dose response over a wide range of doses (100/50 to 12,000 ppm); (2) there was no statistical significance; (3) there were only adenomas, one in each of two doses for females and only one at the high dose in males; (4) the high dose in both male and females were considered excessively toxic; and (5) these tumors occurred in section 5 where there was little to no evidence of non-neoplastic lesions in the nasal mucosa. On the other hand: (1) an adenoma of the respiratory epithelium was seen in one female at 6000 ppm (not an excessive dose); (2) spontaneous nasal tumors are very rare in rats, there were no nasal tumors in the concurrent controls and the incidences exceeded the historical control incidence of the testing laboratory and NTP. The CARC also concluded that for males, the biological significance of the single olfactory epithelial tumor at 6000 ppm is unknown, since it is from a different cell of origin (esthesioneural epithelial neoplasm) and this type of tumor should not be combined with nasal respiratory epithelial neoplasms.

C. Oral Cavity Tumors

In **male rats**, there was one **squamous cell papilloma** of the palate at 100/50 ppm compared to zero in all other groups, including controls. In **female rats,** there was a **squamous cell carcinoma** of the alveolus of the tooth at 100/50 ppm, a **squamous cell papilloma** of the palate at 6000 ppm and a **squamous cell carcinoma** of the palate at 12,000 ppm compared to zero of all three tumor types in the controls. There is considerable uncertainty however, as to the actual incidence of these tumors and how many animals had this tissue examined since the oral mucosa was not considered a routine tissue for histologic examination. It is possible that oral tissue was examined and found negative but results were

not recorded because examination was not part of the protocol.

The single occurrence of a low dose tumor in males was considered to be incidental background since there were no tumors at the higher doses, even with the large dose spread from 100/50 to 12,000 ppm. For females however, the incidence of oral squamous cell tumors in this study (1 at 6000 ppm and 1 at 12,000 ppm) exceeded the historical control incidence from inhalation studies at the testing facility (0/240 males and 0/240 females). In addition, the NTP (1998 report) reported: squamous cell papilloma - females 2/901 (0.22%), squamous cell carcinoma - females 0/901 (0%).

It was difficult to judge the significance of the low dose alveolar tumor since the oral cavity was not routinely examined in this study and the tumor was only seen in one low dose female. Of the two oral palate tumors, one at each of the two high doses, only the one adenoma in the 6000 ppm female was at a dose that was not considered excessive.

The Committee concluded that it could not determine whether the oral cavity tumors in females were treatment-related or due to random occurrence. On the one hand: (1) there was no dose response over a wide range of doses (100/50 to 12,000 ppm); (2) there was no statistical significance; (3) the high dose in the females was considered excessively toxic. On the other hand: (1) a squamous cell papilloma of the palate was seen in one female at 6000 ppm (not an excessive dose); (2) spontaneous oral tumors are very rare in rats, there were no oral tumors in the concurrent controls and the incidences exceeded the historical control incidence of the testing laboratory and NTP; (3) due to the lack of systematic pathologic evaluation of the oral mucosa, there is uncertainty as to the actual incidence of oral tumors. However the CARC determined that a recut would not alter their conclusion.

D. Other Tumors

The Committee concluded that the following tumors are not treatment related for the following reasons:

Male rats - 1) **thyroid gland (follicular cell)** - there was neither statistical (other than a positive trend for combined adenomas and carcinomas) nor biological significance for any tumor type. **Although there was no evidence that the above tumors are treatment related in rats at any dose level, the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12,000 ppm, where mortality was 74% and 100%, respectively. There is, however, no evidence to either support or refute this supposition.**
2) **thyroid gland (C-cell)** - there was neither statistical (other that carcinomas in the 500 ppm group) nor biological significance, there was no dose-response relationship, and the combined tumor incidences in treated groups were comparable to those seen in the concurrent control group.

3) **testes (interstitial cell)** - tumor incidences of this nonfatal tumor were approaching 100% in all groups including controls, and positive statistical significance was considered to be an artifact in the Peto's Prevalence Analyses due to high mortality rather than biological significance.
4) **liver** - there was neither statistical nor biological significance and there was no dose-response relationship. **Although there was no evidence that the above tumors were treatment related in rats at any dose level, the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12,000 ppm, where mortality was 74% and 100%, respectively. There is, however, no evidence to either support or refute this supposition.**
5) **mononuclear cell leukemia** (MCL) - there was no indication of increased incidence or early onset, this tumor occurs commonly in Fischer 344 rats, the incidences were within historical control ranges, there was no statistical significance at any dose, and there was no dose response. Further more: (a) the CARC considered attributing the cause of death to MCL as subjective and not a reliable indicator of increased severity of this tumor; (b) using the incidence of deaths in leukemic animals caused by MCL as a measure of severity is not reliable because establishing a cause of death is subjective in older rats with possible multiple aging processes.

Female rats - 6) **pituitary gland (par distalis)** - the tumor incidences and types in treated groups were comparable to those seen in the concurrent control group; there was neither statistical nor biological significance; and there was no dose-response relationship.
7) **uterus (various types)** - the individual tumor incidences were low, the tumor incidences and types in treated groups were comparable to those seen in the concurrent control group; there was neither statistical nor biological significance; and there was no dose-response relationship.

2. Mutagenicity

Results of the guideline genetic toxicology studies with malathion indicated that the test material did not cause gene mutations in bacteria or UDS in cultured rat hepatocytes. Similarly, malathion was neither clastogenic nor aneugenic up to doses that showed clear cytotoxicity for the target tissue *in vivo*. The CARC included that *in vitro* and *in vivo* findings from the open literature should be interpreted with caution since positive results were seen at cytotoxic doses and/or the types of induced aberrations were asymmetric and, therefore, not consistent with cell survival. The question of test material was also an issue. Although the structure of malathion suggests electrophilicity, **the Committee concluded that the weight of the evidence supports neither a mutagenic hazard nor a role for mutagenicity in the carcinogenicity associated with malathion.**

3. Structure Activity Relationship

Malaoxon, the active cholinesterase inhibiting metabolite of malathion, was not carcinogenic in male or female rats when tested at doses that were judged to be adequate to

assess its carcinogenic potential in the 1996 study. MCL was not considered to be treatment related since statistical significance was seen only in males at a dose that was determined to be excessive, there was no dose-response, and the incidences were within the historical control range of the testing laboratory. Mutagenicity studies published in the open literature indicate that malaoxon was non-mutagenic in bacteria, was not clastogenic in cultured CHO cells, but did produce positive results without metabolic activation in the mouse lymphoma assay and caused sister chromatid exchanges in CHO cells in the absence of metabolic activation. Malaoxon has a structure similar to malathion; hence, the possibility of electrophilicity also applies to malaoxon. Nevertheless, malaoxon is not carcinogenic in male or female Fischer 344 rats.

IX. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (July 1999), the Committee classified malathion as **"suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential"** by all routes of exposure. This classification was based on the following factors:

(i) occurrence of liver tumors in male and female B6C3F1 mice and in female Fischer 344 rats only at excessive doses (statistically significant and outside historical control);
(ii) the presence of a few rare tumors, oral palate mucosa in females and nasal respiratory epithelium in male and female Fischer 344 rats. With the exception of one nasal and one oral tumor in female rats, all other tumor types were determined to occur at excessive doses or were unrelated to treatment with malathion. These tumors can not be distinguished as either treatment related or due to random occurrence;
(iii) the evidence for mutagenicity is not supportive of a mutagenic concern in carcinogenicity; and
(iv) malaoxon, a structurally related chemical, is not carcinogenic in male or female Fischer 344 rats.

The "suggestive" classification was supported by eleven out of sixteen CARC members present at the meeting. Four of the sixteen members of the CARC present at the meeting, thought that the evidence for malathion's cancer potential was weaker than a "suggestive" classification. There were two votes for, "data are inadequate for an assessment of human carcinogenic potential" and two votes for "not likely to be carcinogenic to humans." These opinions were based, in part, on the consideration that: 1) the increase in liver tumors was due to hepatocellular adenomas (benign tumors); 2) there was no statistical significance at non-excessive doses (significance only in the presence of excessive toxicity); 3) the oral and nasal tumors were not considered treatment-related. In addition, they believed that the dose range for malathion's cancer effects was well defined and limited to excessive or near excessive doses. One member abstained.

X. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Quantitative risk assessment for carcinogenicity is not required since the Committee classified malathion as having suggestive evidence for cancer. A cancer dose-response assessment, e.g. a low dose linear extrapolation model, is not indicated for pesticides in the "suggestive" category.

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Members In Attendance At the Previous Cancer Assessment Review Committee Meetings

September 24, 1997:

Karl Baetcke, Luke Brennecke (Pathology Consultant), William Burnam (Chairman), Marion Copley, Virginia Dobozy, Pam Hurley, Mike Ioannou, Nancy McCarroll, Hugh Pettigrew, Esther Rinde, Jess Rowland (Executive Secretary), Joycelyn Stewart, Linda Taylor, and Yin-Tak Woo. Also present were Alberto Protzel, Branch Senior Scientist, Toxicology Branch 1 and Mr. Richard Brown, of Institute for Individual and Organizational Excellence. Data were presented by Brian Dementi, Toxicology Branch 1.

October 8, 1997:

Karl Baetcke, William Burnam (Chairman), Marion Copley, Vicki Dellarco, Richard Hill, Pam Hurley, Mike Ioannou, Nancy McCarroll, Jess Rowland (Executive Secretary), Joycelyn Stewart, Linda Taylor, and Yin-Tak Woo. Also present were Alberto Protzel, Branch Senior Scientist, Toxicology Branch 1 and Mr.Richard Brown, Institute for Individual and Organizational Excellence. Data were presented by Brian Dementi, Toxicology Branch 1**.**

October 15, 1997:

Karl Baetcke, Luke Brennecke (Pathology Consultant), William Burnam (Chairman), Marion Copley, Pam Hurley, Mike Ioannou, Nancy McCarroll, Hugh Pettigrew, Jess Rowland (Executive Secretary), Joycelyn Stewart, Linda Taylor, and Yin-Tak Woo. Also present were Alberto Protzel, Branch Senior Scientist, Toxicology Branch 1. Data was presented by Brian Dementi, Toxicology Branch 1**.**

June 10, 1998:

Karl Baetcke, Luke Brennecke (Pathology Consultant), William Burnam (Chairman), Marion Copley, Virginia Dobozy, Mike Ioannou, Hugh Pettigrew, Esther Rinde, Jess Rowland (Executive Secretary), Joycelyn Stewart, and Linda Taylor. Also present were Edward Budd, Toxicologist, Registration Action Branch 2; Lori Brunsman, Science Analysis Branch, Diana Locke Risk Assessor, Reregistration Branch 2; and Alberto Protzel, Branch Senior Scientist, Toxicology Branch 1. Data were presented by Brian Dementi, Toxicology Branch 1.

February 24, 1999:

Luke Brennecke (Pathology Consultant), William Burnam (Chairman), Marion Copley, Sanju Diwan (Executive Secretary), Virginia Dobozy, Mike Ioannou, Esther Rinde, Jess Rowland, Joycelyn Stewart, Clark Swentzel, and Linda Taylor. Also present were Lori Brunsman and Brenda Tarplee, Science Analysis Branch; Paula Deschamp, Risk Assessor, Reregistration Branch 2; Randolph Perfetti, Associate Director. Data were presented by Brian Dementi, Toxicology Branch 1**.**

June 23, 1999:

Karl Baetcke, Luke Brennecke (Pathology Consultant), William Burnam (Chairman), Vicki Dellarco, Sanju Diwan, (Executive Secretary) Virginia Dobozy, Mike Ioannou, Nancy McCarroll, Esther Rinde, Jess Rowland, Joycelyn Stewart, Clark Swentzel, and Linda Taylor. Also present were Lori Brunsman, Science Analysis Branch and Brenda Tarplee, Science Analysis Branch. Data were presented by Brian Dementi, Toxicology Branch 1.

September 20, 1999:

At this meeting, the following members reviwed the Draft Report dated September 20, 199. *Karl Baetcke, William Burnam (Chairman), Marion Copley, Sanju Diwan (Executive Secretary,) Virginia Dobozy, Mike Ioannou, Nancy McCarroll, Jess Rowland, Clark Swentzel, and Linda Taylor.* Non-members who participated in the review were Lori Brunsman, Science Analysis Branch and Brian Dementi, Toxicology Branch 1. Also present was Paula Deschamp (observer), Risk Assessor of Reregistration Branch 2.

October 4, 1999:

This meeting continued the review of the Draft Report dated September 20, 199. *Karl Baetcke, William Burnam (Chairman), Marion Copley, Virginia Dobozy, Mike Ioannou, Jess Rowland, Clark Swentzel, and Linda Taylor.* Non-members who participated in the review were Lori Brunsman, Science Analysis Branch and Brian Dementi, Toxicology Branch 1. Also present was Paula Deschamp (observer), Risk Assessor of Reregistration Branch 2.

Chronology of the Cancer Assessment Review Committee Meetings

A chronology of the six meetings presented in the 2-February-2000 CARC document as well as the most recent meeting of 12-April-2000 along with a summary of the conclusions reached at each meeting are presented below.

September 24, 1997

The Cancer Assessment Review Committee (CARC) reviewed and evaluated the non-neoplastic and neoplastic lesions of the liver as well as the adequacy of the dose levels tested in the carcinogenicity study in B6C3F1 mice. The CARC also reviewed and evaluated the liver and the nasal tumors, and the adequacy of the dose levels tested in the combined chronic toxicity/carcinogenicity study in rats.

> *The Committee concluded that the liver pathology slides from male mice at all dose levels should be re-evaluated and referred to a pathology work group (PWG) and the PWG evaluation should be done in compliance with the August 24, 1994 Pesticide Regulation Notice 94-5. The Committee also concluded that an assessment on the relevancy of the nasal tumors to treatment could not be completed at this meeting because of the need for a re-evaluation of the nasal tissues from all animals.*

October 8, 1997

The Committee re-assessed the adequacy of the dose levels tested in the combined chronic toxicity/carcinogenicity study in Fischer 344 rats and evaluated the testicular (males), thyroid (males), and the pituitary (females) tumors observed in this study.

> *Due to lengthy discussions and lack of time, the Committee decided to continue the discussion the following week on October 15, 1997.*

October 15, 1997

The Committee re-evaluated the pituitary tumors and continued the review and evaluation of the uterine tumors and mononuclear cell leukemia in female rats. The Committee reviewed and evaluated the non-neoplastic and neoplastic lesions as well as the adequacy of the dose levels tested, in the carcinogenicity study with malaoxon in Fischer 344 rats.

The Committee concluded that a re-evaluation of the following tissues/slides was required in order to ascertain the relevance of the tumors seen in these organs/tissues to treatment.

Species	*Tissue/Slides*	*Dose Levels*
Mouse	*Nasal Turbinate*	*All Animals / all Doses*
Mouse	*Liver*	*Males - All Doses*
Rat	*Nasal Turbinate*	*All Animals / All Doses*
Rat	*Pituitary glands*	*All Females / All Doses*
Rat	*Uterus*	*All Females / All Doses*

The Committee also concluded that a definitive classification on the carcinogenic potential of malathion could not be made at that time due to the need for the re-evaluation of the tissues/slides listed above, but the available data indicate suggestive evidence of carcinogenicity. The Committee also concluded that there are no compelling reasons to deviate from the current linear low-dose approach (Q_1) for human risk characterization (i.e., status quo). However, the method for quantification would be re-assessed after review and evaluation of the requested pathology data.*

June 10, 1998

The Committee evaluated the conclusions reached by the pathology working group (PWG) in their review of liver pathology slides from the carcinogenicity study in male mice (as requested at the 9/ 24/97 meeting) and discussed whether HED should continue to use the existing linear low dose approach (Q_1*) for risk assessments based on *all* liver tumors seen at *all* dose levels in female mice.

The Committee accepted the PWG report on the re-read and concluded that the use of the existing Q_1 for risk assessments should continue since the re-read of the male mouse liver tumors did not provide any compelling reasons to change from the use of the linear low dose extrapolation for human risk characterization.*

February 24, 1999

The Committee continued with the review and evaluation of the re-examination of the following tissues/slides: liver, male mice based on the PWG re-read; nasal tumors, all mice; nasal, thyroid, pituitary and uterine tumors of rats (as requested at the October 15, 1997 meeting).

The Committee concluded that the following additional information and/or data analyses were required:

- ***Nasal tumors - Rat:*** *Independent review of re read data submitted to the Agency (and associated analyses by the EPA reviewer) is required. Only a letter (from Dr. James*

Swenberg) was made available to the Committee members. There was also a discrepancy between the Study Pathologist in the Original Report (listed as Dr. William Wooding) and in the letter from Dr. Swenberg (listed as Dr. Henry Bolte).

Tooth Tumor - Rat: *Re-evaluation of the "diagnosis" of the tumor morphology.*

- ***Thyroid C-cell Tumor - Male Rat:*** *Statistical (Peto's prevalence test) analyses of tumor incidences by SAB/HED and historical control data from the testing laboratory.*
- ***Mononuclear Cell Leukemia - Male Rat:*** *Statistical (Peto's prevalence test) analyses of tumor incidences and historical control data from the testing laboratory.*

June 23, 1999

The Committee evaluated the additional information and/or data requested at the February 24, 1999 meeting.

The conclusions are presented in this report. In summary, the Committee classified malathion as a "likely human carcinogen" and recommended a linear low-dose approach for human risk characterization.

September 20, 1999

The Committee reviewed the Draft -Cancer Assessment Document, dated September 20, 1999

October 4, 1999

The Committee continued the review of the Draft -Cancer Assessment Document, dated September 20, 1999.

April 12, 2000

The Committee met to evaluate: 1) a new Pathology Working Group (PWG) report on the female Fischer 344 rat liver tumors; 2) two issues raised by Dr. Dementi regarding the evaluation of malathion (mononuclear cell leukemia in Fischer 344 male rats and oral tumors in Fischer 344 female rats; 3) the 29-March-2000 letter from Jellinek, Schwartz & Connally, Inc. to Patricia Moe, Re: Comments on EPA's Risk Assessments for Malathion; 4) discuss the weight of evidence and cancer classification for malathion based on the previously listed information. Also included are: revisions due to inconsistencies or errors identified in the CARC #1 report; and references to minority opinions expressed by Dr. Brian Dementi.

The CARC #2 report is a combined summary of the 2-February-2000 CARC (CARC #1) report and the CARC meeting of 12-April-2000. In summary, the Committee classified malathion as "suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential." Quantitative risk assessment for carcinogenicity is not required since the Committee classified malathion as having suggestive evidence for cancer. A

cancer dose-response assessment, e.g. a low dose linear extrapolation model, is not indicated for pesticides in the "suggestive" category. This report supercedes the 2-February-2000 CARC report.

XII. ATTACHMENTS (Although 1-22 are the same references as in the 2-February-2000 CARC document, the document are the same but the citations have been modified to better reflect their content.)

1 Dementi, B.(1997). Untitled memorandum to William Burnam, Chairman, Cancer Assessment Review Committee, dated November 26, 1997. 4 p.

2 Dementi, B.(1998). *"Comments on your draft note to Judy Hauswirth/JSC regarding the meeting of February 18 in your office"*: memorandum to William Burnam, dated February 23, 1998. 1 p.

3 Dementi, B.(1998). Untitled memorandum to Mike Ioannou and William Burnam, dated April 9, 1998. 3 p.

4 Dementi, B.(1998). Letter to Jerry Hardisty, D.V.M., of Experimental Pathology Laboratories, Inc., dated May 4, 1998. 3 p.

5 Dementi, B.(1998). Untitled memorandum to William Burnam, Chairman, Cancer Assessment Review Committee, dated May 29, 1998. 4 p.

6 Dementi, B.(1999). *"Supplemental Information for the Cancer Assessment Review Committee Meeting Scheduled for February 24, 1999 to Resume Evaluation of the Malathion Carcinogenicity Data Base"*: memorandum to Sanju Diwan, Executive Secretary, Cancer Assessment Review Committee, dated February 11, 1999. 3 p.

7 Dementi, B.(1999). *"Leukemia - Recommendation To CARC For Further Assessment"*: memorandum dated February 24, 1999. 2 p.

8 Dementi, B.(1999). Untitled memorandum to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated April 1, 1999. 9 p.

9 Dementi, B.(1999). Untitled memorandum to William Burnam, Chairman, Cancer Assessment Review Committee, dated April 27, 1999. 3 p.

10 Dementi, B.(1999). *"Re: May 3, 1999 report 'Addendum to Malathion Qualitative Risk Assessment Based On Fischer 344 Rat Dietary Study'"*: memorandum to William Burnam, Chairman, Cancer Assessment Review Committee, dated May 18, 1999. 2 p.

11 Dementi, B.(1999). *"Re: Malathion combined chronic toxicity/carcinogenicity study in the F344 rat (MRID 43942901), interstitial cell testicular tumors"*: memorandum to William Burnam, Chairman, Cancer Assessment Review Committee, dated June 7, 1999. 8 p.

12 Dementi, B.(1999). Untitled e-mail message to William Burnam, dated June 21, 1999. 2p.

13 Dementi, B.(1999). Untitled memorandum to William Burnam, Chairman, Cancer Assessment Review Committee, dated July 13, 1999. 7 p.

14 Dementi, B.(1999). Untitled memorandum to William Burnam, Chairman, Cancer Assessment Review Committee, dated July 22, 1999. 2 p.

15 Dementi, B.(1999). Untitled memorandum to William Burnam, Chairman, Cancer Assessment Review Committee, dated September 21, 1999. 2 p.

16 Dementi, B.(1999). *"Comments on September 20, 1999 Draft CARC Report on Malathion"*: memorandum to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated October 6, 1999. 7 p.

17 Dementi, B.(1999). *"Comments on October 28, 1999 Draft CARC Report on Malathion"*: memorandum to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated October 28, 1999. 2 p.

18 Dementi, B.(1999). *"Response to your Nov 5 note to CARC concerning my note of Oct 28 to Jess Rowland"*: e-mail message to William Burnam, dated November 12, 1999. 3 p.

19 Dementi, B.(1999). Malathion: Study Pathologist's Responses (MRIDs 44837001; 44970601; 44970501) to Health Effects Division's Questions on the Nasal Tissue Histopathology Re-evaluation (MRID 44782301) of the Combined Chronic Toxicity/Carcinogenicity Study in the F344 Rat (MRID 43942901): memorandum to Patricia Moe and Paula Deschamp, dated November 18, 1999. (*Also* transmitted to William Burnam, Chairman, Cancer Assessment Review Committee, by untitled memorandum dated December 7, 1999.) 4 p.

20 Dementi, B.(2000). *"Re: Malathion memo of December 7, 1999"*: untitled e-mail message to William Burnam, dated January 12, 2000. 4 p.

21 Dementi, B.(2000). Untitled memorandum to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated February 7, 2000. 2 p.

22 Dementi, B.(2000). *"Comments on February 2, 2000 CARC report on malathion"*: memorandum to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated February 9, 2000. 8 p.

23. Dementi, B. (2000). *"Response to Dr. Copley's assessment of malathion CARC issues*". E-mail to William Burnam, dated April 10, 2000. 9 p.

24. Dementi, B. (2000). *"Comments to the Draft April 2000 CANCER ASSESSMENT DOCUMENT #2: Evaluation of the Carcinogenic Potential of Malathion*". E-mail to Marion Copley, dated April 24, 2000. p. 8

25. Copley, M.(2000). *"Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion*": memorandum to William Burnam and Margaret Stasikowski, dated March 30, 2000. 31 p.

26. Copley, M.(2000). *"Responses to Concerns Expressed by Dr. Brian Dementi (memo dated 9-Feb-2000) Regarding the Final HED Cancer Assessment Review Committee Report (2-February-2000) for Malathion"*: memorandum to William Burnam and Margaret Stasikowski, dated April 27, 2000. 13 p.

27. Dementi, Brian (2000) Untitled memorandum addressed to John Carley, Office of the Director, OPP, dated January 27, 2000. 4 p.

28. Dementi, Brian (2000) *"Summary of malathion CARC issues"*: e-mail to John Carley dated February 3, 2000. 6 p.

29. Dementi, Brian (2000) Untitled memorandum addressed to William Burnam, Chairman, Cancer Assessment Review Committee, dated April 27, 2000. 13 p.